VOLUME 19 NUMBER 11

NUTRITION REVIEWS

November 1961

Who Is Well Nourished? by Stanley N. Gershoff 321

Clinical Nutrition

Kwashiorkor in Cape Town 324
Treatment of Hypercholesterolemia with Nicotinic Acid 325
Diet and Atherosclerosis in London Busmen 328

Animal Nutrition

Effect of Excessive Calcium on Selenium 75 Utilization in Swine 330

Experimental Nutrition

Sodium Chloride and Myocardial Infarcts in Rats 331
Dietary Fats and Atherosclerosis and Thrombosis in the Rat 333
Intestinal Conversion of C^{14}-Carotene to Vitamin A 334
Fatty Acids and Riboflavin in the Metabolism of Vitamin B_6 336
Experimental Atherosclerosis in Dogs with Aortic Grafts 338
Cadmium, A Possible Dietary Essential 340
Effect of Excessive Lysine and Leucine Intakes on the Pregnant Rat 342
Vitamin A and Proteins in Blood and Liver 344
Pathologic and Physiologic Studies in Canine Atherosclerosis 346
Trivalent Chromium and Glucose Tolerance 348

Notes

Letter to the Editor 350
Chemicals in Food 351
Dietary Treatment of Familial Mediterranean Fever 352
Recent Books 352

Published Monthly at Mt. Royal and Guilford Aves., Baltimore 2, Md.

For

THE NUTRITION FOUNDATION, INC.

99 PARK AVE. NEW YORK 16, N. Y.

COPYRIGHT 1961 NUTRITION FOUNDATION INC. NEW YORK U.S.A.

Editor

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, One Shattuck Street, Boston, Mass.

Assistant Editor

ADELE GLIMM, One Shattuck Street, Boston.

Associate Editors

GEORGE K. DAVIS, PH.D., Department of Animal Husbandry and Nutrition, University of Florida, Gainesville.
GILBERT B. FORBES, M.D., Department of Pediatrics, University of Rochester School of Medicine, Rochester.
ROBERT E. HODGES, M.D., Department of Internal Medicine, State University of Iowa, Iowa City.
WILLIAM G. HOEKSTRA, PH.D., Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.
STANLEY LEVEY, PH.D., Department of Biochemistry and Nutrition, School of Medicine, University of Southern California, Los Angeles.
OLAF MICKELSEN, PH.D., Laboratory of Biochemistry and Nutrition, National Institute of Arthritis and Metabolic Diseases, Bethesda.
WILLIAM J. PYLES, M.D., St. Luke's Hospital, New York.
JAMES H. SHAW, PH.D., Schools of Dental Medicine and Public Health, Harvard University, Boston.
ELMER H. STOTZ, PH.D., Department of Biochemistry, School of Medicine, University of Rochester.
DONALD M. WATKIN, M.D., Organización Panamericana de la Salud, Mexico City.
ROBERT W. WISSLER, PH.D., M.D., Department of Pathology, University of Chicago.

Editorial Committee

WALLACE R. AYKROYD, M.D., University of London
CHARLES S. DAVIDSON, M.D., Boston City Hospital
CONRAD A. ELVEHJEM, PH.D., University of Wisconsin
IAGO GALDSTON, M.D., New York Academy of Medicine
WENDELL H. GRIFFITH, PH.D., University of California
CHARLES GLEN KING, PH.D., Nutrition Foundation
BERWYN F. MATTISON, M.D., American Public Health Association
L. A. MAYNARD, PH.D., Cornell University
HAZEL STIEBELING, PH.D., Institute of Home Economics, U.S.D.A.
LEROY VORIS, PH.D., National Research Council
WARREN WEAVER, PH.D., Alfred P. Sloan Foundation
JOHN B. YOUMANS, M.D., American Medical Association

Subscriptions should be sent to The Nutrition Foundation, Inc., 99 Park Ave., New York 16, N. Y.
Rate $4.00 per year in the United States and Canada; $4.50 in other countries.
Changes of address should be sent to The Nutrition Foundation.
Second-class postage paid at Baltimore, Md.

Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved.

NUTRITION REVIEWS

VOL. 19 NOVEMBER 1961 No. 11

WHO IS WELL NOURISHED?

"Who Is Well Nourished? How Can You Tell?" was the title of a Diet Conference sponsored by the Nutrition Foundation and the Council on Foods and Nutrition of the American Medical Association at the latter's annual meeting in June, 1961. Clinical, laboratory, anthropometric, and dietary methods of evaluating nutritional status were discussed by Drs. R. Shank, S. Gershoff, J. Brožek, and B. Bosley.

The meeting emphasized the difficulties in determining nutritional status, particularly of individuals as compared to groups of people. It was generally agreed that in assessing the nutritive status of individuals, clinical and dietary appraisals are still the two most frequently used criteria.

Dr. Shank pointed out that the medical history and physical examination of the patient remain the most important tools of the physician in his provision of medical care. A good medical history can provide information which will call for special attention in examining certain areas of the body or will indicate the need for certain types of laboratory procedures. It may provide information concerning disorders which cause loss of nutrients from the body, decreased gastrointestinal absorption or altered nutrient requirements. Less frequently in the United States, the history may afford evidence of symptoms and physical signs of nutrient deficiency.

In order for a physician to determine whether manifestations of nutritional disease are due to inadequate diets or develop secondarily to metabolic and physiological abnormalities, every medical history should record the dietary practices of the patient as well as his food dislikes and intolerances. It is also important to note the use of drugs which may alter nutrient requirements, a recent example being the increased need for vitamin B_6 in individuals receiving isoniazid therapy.

Each physical examination should record the height and weight of the individual. This makes possible an estimate of deviation from optimum weight. With repeated visits to the physician, changes of body weight may provide evidence of developing obesity or loss of weight. Examiners should appraise gross body contours rather critically to obtain evidence of caloric undernutrition or overnutrition.

In discussion, Dr. W. Darby pointed out that in pernicious anemia a patient's slight weight loss may be the first evidence of gastric malignancy. Dr. Shank emphasized that through examination of body surfaces, particularly the skin, eyes, and mucous membranes in and about the oral cavity, evidences of nutritive failure may be found.

In good health the skin is warm, moist, of good turgor, of vibrant color, and free of abnormal pigmentation. Follicular hyperkeratosis is often seen in vitamin A deficiency. In vitamin C deficiency perifolliculitis develops initially in the skin of the thighs just above the knee but later may appear on the buttocks, abdomen, legs, and arms. Other petechial hemorrhages and areas of purpura may evolve in the skin of scorbutics. Purpura also develops in vitamin K deficiency, the result of reduced prothrombin concentrations, which may also be associated with hepatic disease.

Skin and nail beds are pale in all anemias. Nutritional factors of greatest importance in anemias are deficiencies of iron, vitamin B_{12}, and folic acid. Yellow discoloration of the anemic skin should suggest the possibility of pernicious anemia. Peripheral cyanosis and dirty brown splotches of pigmentation often accompany caloric sub-

nutrition. Symmetrical dermatoses are seen in pellagra and seborrheic lesions of the face, particularly in nasolabial folds, may occur in deficiencies of vitamins B_2 (riboflavin) and B_6 (pyridoxine).

Edema occurs in starvation, protein deficiency, thiamine deficiency, liver disease of nutritional origin, and sodium retention.

In the undernourished the sclerae are often dull and lusterless and optic tensions are reduced. Vascularization of the cornea occurs in a variety of deficiency disorders. Xerophthalmia is a manifestation of vitamin A deficiency.

The membranes in and about the mouth reflect a variety of nutritional deficiencies. Cheilosis, angular stomatitis, hypertrophic bleeding gums, glossitis, and hypertrophy and atrophy of the lingual papillae are associated with deficiencies of niacin, riboflavin, ascorbic acid, thiamine, and iron.

Palpation permits other observations of potential nutritional significance. Bradycardia is often associated with caloric undernutrition while tachycardia may indicate anemia or beriberi heart disease. Gynecomastia and hepatomegaly are found in chronic undernutrition or with liver disease of nutritional origin.

The neurologic examination may demonstrate the nutritional neuropathies which occur in vitamin B_1 deficiency, chronic alcoholism, pregnancy, severe intestinal malabsorption, pellagra, and pernicious anemia.

The abnormalities which have been discussed are indicators of possible malnutrition. In their absence it may still not be wholly warranted to assume that a patient is well nourished. The positive indications of good nutrition are vitality, alertness, good muscular development and tone, body weight within ten per cent of desirable weight, and integrity of all body tissues and organ systems permitting physical and mental well being.

Dr. Darby stated that in doubtful cases a therapeutic test will often allow a diagnosis of nutritional deficiency to be established.

Dr. Gershoff pointed out that before clinical signs of nutritional deficiencies appear, there must be biochemical lesions. The problem that faces biochemists is to develop practical, inexpensive laboratory tests which adequately measure these biochemical lesions. Most medical laboratories do not routinely do many of the laboratory tests used in determining nutritional status. In cases of general malnutrition it is likely that multiple deficiencies occur and that physical, dietary, and anthropometric examinations will provide sufficient information to suggest the proper course of treatment.

Physicians interested in public health nutrition find that biochemical data are of considerable value in determining the nutritional status of groups of people. In recent years the U. S. government through its Interdepartmental Committee on Nutrition for National Defense has conducted nutritional surveys in underindustrialized countries all over the world. In general, laboratory tests used in determining nutritional status of populations consist of the measurement in blood or urine of nutrients or products of their metabolism. Such tests may be done with or without load tests of the nutrient being studied. Measurements of enzyme activity or metabolites whose concentration in blood and urine varies with enzyme activity are also done. Hemoglobin, hematocrit, and serum protein levels are also determined. Dr. Gershoff indicated that although these methods are routinely used, many unsolved problems in sampling techniques and analytical methods remain.

Many people, because of inborn errors of metabolism, disease conditions, the use of various pharmaceutical agents or an altered physiological state, have nutritional requirements which deviate from what is considered normal. Laboratory investigation of pathologic conditions which fall into these categories is receiving a major share

of the attention of nutritional biochemists. Studies in recent years of vitamin B_6 metabolism, for example, have indicated that increased requirements for this vitamin may occur during pregnancy, in some cases of seborrheic dermatitis, in individuals treated with isoniazid, in patients with urinary stones, and in a few convulsive infants with inborn errors of metabolism. In individuals such as these, laboratory tests may be very effective as a diagnostic tool.

Dr. Brožek pointed out that the field of anthropometry has been rapidly expanding. Traditionally, anthropometry has involved the measurement of man by the use of scales for the determination of weight; of spreading and sliding calipers for the measurement of widths; of steel tapes, for measuring circumferences; and of skinfold calipers, for determining the width of the layer of subcutaneous fat which accounts for the greater part of the differences, intra- and inter-individual, in the thickness of folds of skin.

New anthropometric techniques are concerned with the analysis of body composition: soft tissue roentgenogrammetry, densitometry (the ratio of mass and volume), hydrometry (analysis of body composition based on measurement of body fluids), and determinations of electrolytes, especially of potassium, using radioactive isotopes.

Unfortunately, at the present time most physicians are still limited to the traditional measurements of gross body weight and external dimensions as anthropometric means of evaluating nutritional status. Much research still remains to be done on the various new anthropometric techniques. Although very useful data on total body fat can be obtained from two skinfold measurements on young men, it is much more difficult to do the same with old men and almost impossible with women. We still are unable to determine the mineral content of bone from x-rays.

In answer to a question, Dr. Brožek pointed out that measurement of thiocyanate space can be very useful in diagnosing edema unaccompanied by pitting.

Dr. Bosley stated that dietary evaluation can be useful in supplementing and supporting other methods used in assessing the level of nutriture. Well-trained nonmedical people usually can do dietary evaluations more efficiently than most physicians. One of the most common methods used is the 24 hour recall of foods eaten. The success of this method depends on the interviewing technique and judgment of the interviewer. If records are obtained over a sufficiently long time or at such intervals as to reflect seasonal differences, they may provide indications of customary dietary habits, food preferences, cultural practices, methods of food preparation, attitudes toward food, and economic status. Some of these factors may be of particular importance in evaluating diets of people living alone or old persons. Records of this type do not normally lend themselves to detailed analyses of nutrients provided. They merely identify the quality of the food eaten.

Quantitative analyses are obtained by actually measuring food consumption of individuals, or in institutional type surveys where the amount of food served to a group and the waste are known, the average serving per individual can be calculated for a specific time.

Poor correlations between dietary evaluations and biochemical or physical examinations often occur. Both biochemical and physical examinations may reflect the dietary patterns of a relatively long period of time. The dietary record may not necessarily reflect past practices. By themselves dietary evaluations may not provide an accurate appraisal of nutritional status but in combination with other methods of evaluation they can make a significant contribution to determining whether or not we are well nourished.

STANLEY N. GERSHOFF, PH.D.
Department of Nutrition
Harvard School of Public Health
Boston, Massachusetts

KWASHIORKOR IN CAPE TOWN

A series of 150 patients hospitalized for kwashiorkor was studied. A follow-up study revealed the progress of one-third to be unsatisfactory six to 18 months after return home.

It is becoming increasingly apparent that kwashiorkor is a disease involving the interaction of multiple etiologic factors. No longer is it possible to think of this condition solely in terms of protein-calorie undernutrition. A more realistic approach must ask why some children in underdeveloped areas develop the disease while others escape. Also, why do many children fail to receive adequate amounts of protein? These and many other questions are now receiving increased emphasis.

A number of these questions are discussed by A. Moodie (*J. Pediat.* **58,** *392* (*1961*)). They are further emphasized by the follow-up study made of a series of patients treated in a hospital for kwashiorkor.

The author studied a series of 150 patients in Cape Town. The mother was interviewed at the time of the child's admission to the hospital. In addition, a home visit was made. Assessment of the health status and mode of rearing of the siblings and relatives was also made. In this way comparisons could be made between patients and those children in the community who did not have kwashiorkor.

Follow-up observations were made on 132 children from one to 18 months after discharge from the hospital. At this time the children were weighed and measured, and both their general condition and the family situation were noted.

The duration of breast feeding was determined for the patients and their siblings. Fifty-three per cent of the patients had been weaned from the breast by three months of age and 67 per cent by six months. Comparative figures for the siblings were 39 per cent and 45 per cent, respectively. Reasons given for early weaning included such factors as illness of the mother or her return to work in 11 per cent, and "inadequate milk" supply in 62 per cent. It is unfortunate that in this setting, with so many factors conducive to kwashiorkor, more infants are not breast fed, and for longer periods of time.

The growth performance of siblings and neighbor children was compared to published data (G. M. Lurie and F. J. Ford, *South African Med. J.* **32,** *1017* (*1958*); E. P. Woodrow and I. Robertson, *Ibid* **24,** *761* (*1950*)) on Cape Town infants and children. Plots of heights and weights revealed that the majority of these "controls" fell below the mean of the population. This was true for both boys and girls and over an age span of one and one-half to 12 years. It is apparent that a certain degree of malnutrition is widespread among the population under study.

The role of infection in precipitating the onset of clinical kwashiorkor has been pointed out many times (N. S. Scrimshaw and M. Béhar, *Science* **133,** *2039* (*1961*)). Moodie found that 73 per cent of the patients in her study had had diarrhea in contrast to 15 per cent of the "controls." It is difficult to evaluate this difference, however, since diarrhea is such a frequent symptom of the disease itself. Eight per cent of patients had had recent measles and 6 per cent pneumonia. No statements are made about other infections such as intestinal parasitism, malaria, and others known to be prevalent in this area.

Feeding preferences are much the same as in other areas where kwashiorkor flourishes. Low protein foods, or those in which protein quality is poor, are commonly given. Many mothers have the feeling that meat is not a suitable food, despite the fact that they recognize that their children will eat meat readily when it is offered. About two-thirds of the children receive no milk or a negligible amount.

Social and economic factors lie at the

heart of the matter. From the point of view of hygiene, 90 per cent of the homes were judged unsatisfactory. Forty per cent of the fathers were unemployed, and 80 per cent of the families were subsisting on incomes below that which could provide a minimum satisfactory living standard. An estimate of family income at the time when the sibling "controls" were young indicated considerably less poverty. The illegitimacy rate is about twice as high among these people as it is for the Cape Colored population in general. Forty per cent of mothers worked outside the home.

Attendance at child welfare clinics was sporadic (though less so in "controls" than in patients) and ignorance of matters of child care and feeding was widespread.

What happens to the kwashiorkor patient after he has been treated in the hospital and returns to his home environment? A follow-up evaluation was made at intervals up to 12 to 18 months in 31 patients, up to six to nine months in 47 patients. The remainder were followed for shorter periods of time.

Of the total group of 132 patients, 8 per cent had died—four from recurrent kwashiorkor, four from infection. An additional 10 per cent had recurrence of kwashiorkor from which they recovered. Sixteen per cent were not gaining weight in a satisfactory fashion. Therefore, in almost one-third of the patients progress was clearly unsatisfactory. Perhaps this result is to be expected, since the children returned from the hospital to the same environment in which they originally developed the disease.

The author does not state the type of treatment given in the hospital or the duration of stay there. It would be interesting to know whether the group who did fairly well after discharge from the hospital was handled in the same way in the hospital as those who did not.

The author concludes that social, economic, and cultural factors, combined with ignorance, are basic to this problem, and that "the basic remedy for malnutrition in Cape Town must be an economic one."

Many of the families involved in her study had recently migrated to Cape Town from rural areas to seek work in industry.

One cannot help but be reminded of the situation in regard to infant mortality during the early phases of the industrial revolution. The appallingly high infant death rates in the newly industrialized cities of eighteenth century Western Europe can be traced, in part, to the same factors now operating to foster kwashiorkor among the admittedly more primitive peoples of Africa. Crowding, family disruption, poor hygiene, recurrent unemployment, poverty, ignorance—all of these are common to both situations. However, the fact that kwashiorkor exists in rural areas of Africa as well as in recently urbanized groups, renders the problem more difficult. And what is even more of a handicap for the rehabilitationist is the cultural barrier which exists between the victims of the disease and those who have the knowledge of its cause and cure.

TREATMENT OF HYPERCHOLESTEROLEMIA WITH NICOTINIC ACID

Large doses of nicotinic acid administered orally have resulted in significant reduction in the levels of serum cholesterol and of beta lipoprotein cholesterol. A few patients developed diabetes mellitus or hepatic dysfunction after several years of treatment.

The quest for effective means of reducing human hypercholesterolemia continues. Although most investigators realize that decreasing the amount of cholesterol in the serum is a clinical experiment, few of them doubt the wisdom of gaining more experience with the use of hypocholesterolemic agents. The level of cholesterol in the serum can be

lowered in many ways. The most desirable, from the standpoint of a patient, would be with an agent which not only allowed him to continue his customary diet but was effective, safe, inexpensive, and free from unpleasant reactions. So far no substance has fulfilled all of these criteria.

The use of nicotinic acid in large doses to lower the level of cholesterol in the serum has been reviewed many times (*Nutrition Reviews* **17,** *78, 168, 291 (1959)*; **18,** *174, 342 (1960)*). These accounts have indicated that nicotinic acid, to be effective, must be given in massive doses of 3 g. or more daily.

W. B. Parsons, Jr. has studied the clinical application of nicotinic acid to hypercholesterolemic patients and has followed his subjects for a long time. In two reports he summarized both the efficacy of this form of therapy (*Arch. Int. Med.* **107,** *639 (1961)*) and its potential toxic effects (*Ibid*, **107,** *653 (1961)*). Parsons reviewed the reasons for using nicotinic acid to suppress the level of serum cholesterol. In large doses it reduces not only free and esterified cholesterol (H. Gurian and D. Adlersberg, *Am. J. Med. Sci.* **237,** *12 (1959)*), but also the beta lipoprotein cholesterol fraction (Parsons *et al.*, *Proc. Mayo Clinic* **31,** *377 (1956)*); Parsons and J. H. Flinn, *Arch. Int. Med.* **103,** *783 (1959)*). It is this fraction which has been cited by R. E. Olson to be most suspect in the production of vascular atheromata (*Circulation* **22,** *453 (1960)*).

In the present report Parsons summarized his experiences since 1956. Fifty patients were included by virtue of having completed one year or more of therapy. The patients were divided into two groups. The first group included 26 who were selected during the first year of the study because they had an average level of serum cholesterol greater than 270 mg. per 100 ml. The second group of 24 comprised all others added to the study. Seven of these had cholesterol values less than 270 mg. per 100 ml., but had abnormalities of their beta lipoprotein cholesterol fraction or of the ratio of beta lipoprotein cholesterol to alpha$_1$ lipoprotein cholesterol. Thirty-five subjects were men averaging 48 years of age and 15 were women averaging 50 years of age. Previous myocardial infarction had occurred in 17 patients, angina pectoris was present in four, arteriosclerosis obliterans in four, and cerebral infarction in four. Four patients had essential hypertension and three had xanthelasmata. One had xanthoma tendinosum. No vascular disease was evident in 13 patients. Two additional subjects had xanthoma tuberosum of the skin and extremely high levels of serum cholesterol. These were reported separately.

All of the patients remained ambulatory and ate their usual diets at home. Nicotinic acid was given in a dose of 3 g. orally per day in divided doses for the first 12 weeks. If a satisfactory response occurred this dose was continued. If not, the dose was increased to 4.5 g. per day. After another six to eight weeks, if improvement failed to occur, the dose was increased to 6 g. and later 7.5 g. daily. In recent years modified preparations of nicotinic acid were substituted for the original drug in a few instances.

Laboratory studies included determinations of the amount of cholesterol and alpha$_1$ and beta lipoprotein cholesterol fractions in their serum. Annually the author obtained a complete evaluation of each patient which included blood counts, urinalyses, blood glucose, non-protein nitrogen, sulfobromophthalein test, bilirubin, cephalin flocculation, thymol turbidity, alkaline phosphatase, and serum glutamic-oxaloacetic transaminase. Total protein and protein electrophoresis were performed; in a number of instances hepatic biopsies were performed.

The results of the first 56 weeks of treatment were quite remarkable. Patients in

the first group had an average reduction of 60 mg. per 100 ml. in their serum cholesterol concentration by the end of the first 12 weeks and a further reduction of 75 mg. per 100 ml. by the end of 30 weeks. The subjects in the second group had decreases of about the same magnitude. When nicotinamide was substituted for nicotinic acid the level of cholesterol in the serum rose to pretreatment values but responded quickly when nicotinic acid therapy was resumed. Changes in beta lipoprotein cholesterol paralleled those of cholesterol. At the same time, the $alpha_1$ lipoprotein cholesterol fraction increased, indicating a shift in cholesterol transport mechanism. For statistical purposes the author considered the upper limit of normal values to be 250 mg. per 100 ml. for cholesterol, 180 mg. per 100 ml. for beta lipoprotein cholesterol, and the ratio of 4.0 for the beta to $alpha_1$ lipoprotein cholesterol. Individual reductions of 40 mg. per 100 ml. or more in cholesterol or in beta lipoprotein cholesterol were considered significant.

Of the 50 patients, 40 attained normal levels for both cholesterol and beta lipoprotein cholesterol. Three more had significant decreases in cholesterol and normal levels of lipoprotein cholesterol. Six others had significant reductions of both fractions, but not to normal levels. Only one patient failed to have any response. There was no tendency for "escape" from the effects of therapy. In every instance, cessation of therapy or substitution of a placebo or of nicotinamide resulted in a prompt reversal of these effects.

Since the primary reason for regulating cholesterol metabolism is to prevent deposition in vascular tissue, the effects on prognosis must await the passage of time. Studies in rabbits indicate that nicotinic acid has some protective action against experimental atherosclerosis (R. Altschul, *Z. Kreislaufforsch.* **45,** *573 (1956)*; E. E. Cava *et al.*, *Proc. Mayo Clinic* **34,** *502 (1959)*; J. M. Merrill and J. Lemley-Stone, *Circulation Research* **5,** *617 (1957)*).

The two patients with extremely high levels of serum cholesterol and with xanthoma tuberosum had spectacular responses to therapy. The first had typical lesions around the elbows and knees. His serum cholesterol, which initially was over 900 mg. per 100 ml., declined to less than 150 mg. per 100 ml. within six months. By 14 months the xanthomata were gone. The second patient also had large xanthomata of the elbows. His serum cholesterol declined from about 700 mg. per 100 ml. to approximately 150 mg. per 100 ml. and after six months his xanthomata were softer and smaller.

Annoying side effects from nicotinic acid therapy consist of cutaneous flushing, gastrointestinal symptoms, and cutaneous changes. Most patients developed tolerance to the sensation of flushing but in those who failed to do so the substitution of a delayed release preparation lessened or eliminated this symptom. Gastrointestinal distress consisting of heartburn, and in a few patients, nausea, did not seem to be lessened by using delayed release forms. Seven patients developed activation of peptic ulcer (Parsons, *J. Am. Med. Assn.* **173,** *1466 (1960)*). Mild diarrhea occurred in three patients.

As might be expected, some patients attributed changes in various symptoms to the medication. Although several felt that chest pain was lessened, arthralgias relieved, and sexual potency altered, the author doubted that any of these changes were of significant magnitude.

Many patients in the author's series complained of dryness of the skin. Four developed localized roughness and pigmentation suggestive of acanthosis nigricans. The author speculated that such alterations might indicate changes in cholesterol metabolism.

Other metabolic effects indicated altera-

tions in hepatic function and impairment of glucose tolerance. In most cases hepatic dysfunction seemed to be reversible and presumably related to enzymatic reactions. During the first year of therapy the author found no significant evidence of hepatic damage. However, beginning in the second year of the study, eight patients showed significant sulfobromophthalein retention in two consecutive tests at least a week apart. Four of these underwent needle biopsy of the liver. The findings in these varied from marked cloudy swelling to fatty metamorphosis, inflammatory infiltration, and in one instance, fibrosis and cholangiolitic involvement. One of these subjects developed diabetes mellitus. The author felt that patients who were given a slowly absorbing form of nicotinic acid were more apt to have hepatic dysfunction, perhaps because of the continuous blood levels of the drug.

The glucose tolerance curve was altered in many patients taking nicotinic acid. Only three of the patients in this series developed frank diabetes mellitus and in these it was easily controlled. The levels of serum uric acid were increased slightly but no clinical symptoms could be attributed to this effect.

Nicotinic acid has been reported to activate fibrinolysis (M. Weiner, K. deCrinis, W. Redisch, and J. M. Steele, *Circulation* **19,** *845 (1959)*). Although the author confirmed this report when he gave nicotinic acid intravenously, the effect was lacking when the drug was given orally despite the fact that higher blood levels were achieved. Repetition resulted in loss of this response.

The author discussed, in considerable detail, possible mechanisms of action whereby nicotinic acid might alter cholesterol metabolism. They include 1) vitamin effects, 2) direct relationship to cutaneous flushing, 3) competition for methyl groups, 4) competition for glycine, 5) presence of nicotinuric acid, 6) decreased fatty acid synthesis and increased cholesterol synthesis in the liver, 7) decreased cholesterol synthesis, 8) increased oxidation of cholesterol, 9) subclinical anorexia, and 10) artifact. The author considered the mechanism to be an open question, but he felt that tenable ideas included alterations in cholesterol synthesis and increased intrahepatic oxidation of cholesterol.

These studies are of great interest to investigators, not only because of potential clinical application, but because through them the chemistry and metabolism of cholesterol can be better understood. It is apparent that this method of treating hypercholesterolemic patients, like all others, is an experiment in which the patient must participate with the investigator. The possibility of inducing permanent diabetes mellitus or permanent hepatic dysfunction must be considered. The author has done an admirable job of collecting data, interpreting it conservatively, and studying his problem in sufficient depth to warn others of potential hazards.

DIET AND ATHEROSCLEROSIS IN LONDON BUSMEN

There was no difference in food intake between London bus drivers and conductors, even though the former had a much higher incidence of cardiovascular deaths.

The work of J. N. Morris and co-workers (*Nutrition Reviews* **16,** *129 (1958)*; **18,** *267 (1960)*) revealed a higher incidence and greater severity of coronary heart disease among the drivers than among the conductors of London buses. These investigators suggested that the difference in physical activity of the two groups was partly

responsible for the observed variations in incidence of coronary heart disease.

As part of the above studies, E. M. Bramwell carried out a dietary survey among 34 drivers and 30 conductors (*Proc. Nut. Soc.* (*London*) **20,** *30* (*1961*)). These men ranged in age from 34 to 49 years and presumably worked in double-decker buses.

Each man kept a log of all food and drink consumed during seven consecutive days. During this week, all food eaten at home was weighed, as was the food eaten in the company cafeteria. During the week when the food records were kept, all men worked the shift starting at 2 p.m. One or two free days were included in the period. However, some of the men worked another shift on these days.

Most men had food patterns which varied little from day to day. They liked plain foods with little variety. Changes in shifts had little effect on the dietary pattern, except to change meal hours.

There was little difference in the food patterns of the drivers when compared with that of the conductors. For both groups breakfast consisted of such items as bread, cereal, porridge, and tea. The main meal was at midday. It consisted of a meat, fish, egg or cheese dish with vegetables. Many men had either fruit tarts or cooked fruit with custard for dessert. Surprisingly, many of the men had meat pies secured at the cafeteria for snacks. About half the men brought sandwiches for their lunch; these nearly always contained meat, cheese or egg. Cake or fresh fruit was the usual dessert. A third of the men lived close enough to eat their lunch at home. For them, the meal consisted of such things as eggs and bacon or sausages, or occasionally a meat pie or meat and vegetables.

Most men had something to drink on their return home after work, generally tea, milk or beer. A third of them also had a sandwich or eggs and bacon or breakfast cereal.

Meals on Sunday were an elaboration of the weekday meals. Frequently, there were fewer meals but more food was consumed at each meal.

The drink used in largest amount was tea. These men averaged nearly seven cups of tea per day, which is approximately the national average for adults in Great Britain. Alcohol was consumed primarily as beer. The average busman consumed one or two pints of beer per week, which is considerably below the British average of 4.7 pints per adult.

The calculated daily caloric intake of the drivers was 2,850 and that of the conductors 2,790. This diet provided 83 g. of protein and 128 g. of fat, of which 51 g. was from animal sources. There was no essential difference in the composition of the diets of the two groups.

The caloric intake in excess of expenditure partly explains the greater degree of obesity among the drivers when compared with the conductors. That the difference did not develop only as a result of their jobs has been emphasized by Morris (*Arch. Int. Med.* **104,** *903* (*1959*)). The differences in physical constitution of the two groups were the same for men 25 to 29 years of age as for those 55 to 64. These differences were apparent in such measurements as waistlines, body weights, and skinfold thicknesses.

A. Kagan (*Proc. Roy. Soc. Med.* **53,** *18* (*1960*)), working with the same group of men, reported the drivers to be about 8 kg. heavier than the conductors. The sum of the skinfold measurements for the triceps, subscapular, and suprailliac areas was 47 mm. for the drivers and 37 for the conductors. The total plasma cholesterol for the drivers was 250 mg. per 100 ml., while that for the conductors was 238. There was no essential difference in the values for the beta-lipoproteins (large molecular weight compounds which contain about 45 per cent cholesterol) in the plasma, but for all age groups, the Sf 20–400 lipoprotein (contain

less than 25 per cent cholesterol and large amounts of triglycerides) fractions for the conductors was two-thirds that of the drivers.

The similarity in the dietary intakes of the London busmen provides no solution to the observation of Morris *et al.* (*Lancet* **2,** *1053 (1953)*) of an incidence and early mortality rate of coronary disease among the drivers that is two to four times (*Arch. Int. Med.* **104,** *903 (1959)*) greater than for the conductors. It has led the groups involved in this important study to suggest that different types of men as judged by simple anthropometric measurements are attracted to the jobs of drivers and conductors. The greater physical activity of the conductors during working hours may be another factor that reduces their risk of coronary heart disease. The conductors and drivers differ very little in weekly pay, less than five shillings according to Kagan, and in social background.

It would be very helpful if correlations were made between some of the more important parameters being measured. Such information would be more valuable than the simple averages without any statistical limits that have appeared from this study so far.

EFFECT OF EXCESSIVE CALCIUM ON SELENIUM 75 UTILIZATION IN SWINE

Calcium levels as high as 2.4 per cent of the diet did not markedly influence the amount of selenium 75 which was absorbed or deposited in whole organs. Probably because of a depressing effect upon diet consumption, the high level of calcium reduced the selenium content of tissue.

It has been well established that dietary calcium has an influence on many other dietary constituents. It is quite natural, with the increasing attention being given to selenium as a dietary element, that studies should be made of the influence of various levels of calcium in the diet upon the absorption and utilization of selenium. The relatively long half life of selenium 75 (127 days) and the fact that it is a gamma emitter readily permit its use in the study of the metabolism of selenium by animals.

R. G. Buescher, M. C. Bell, and R. K. Barry (*J. Animal Sci.* **20,** *368 (1961)*) have carried out studies with swine in which they fed levels of calcium from 0.6 to 2.4 per cent of the diet, along with oral doses of selenium 75 in the form of selenious acid. This was a classical distribution type of experiment in which they studied the level of selenium in blood, urine, and feces at various intervals up to 120 hours after dosage, and at the termination of the collection period, sacrificed the animals and made determinations of selenium in liver, lung, spleen, heart, and kidney, as well as in such tissues as the small intestine, vertebra, and other bones.

Interestingly enough, the high level of calcium, 2.4 per cent, depressed the appetite of young pigs weighing about 50 pounds, resulting in somewhat lower weight gains for this group. When selenium 75 was administered, these pigs showed a somewhat higher concentration of the selenium in the various tissues.

Buescher, Bell, and Barry recognized the error due to this difference in size and reduced the results to a per organ or per tissue basis, rather than a per gram or per milliliter basis. When these corrections were applied the level of selenium in the blood was essentially the same for all groups treated. The selenium was absorbed very rapidly, reaching a peak at about eight hours after dosage. After 36 to 48 hours it plateaued at a level which remained almost the same through the remainder of the 120 hour collection period.

Approximately 73 per cent of the total blood selenium 75 was found in the plasma. More than 70 per cent of the administered selenium 75 was excreted by way of the feces, which contrasts with reports on the excretion of selenium by rats (K. P. McConnel, *J. Biol. Chem.* **145,** *55 (1942)*). Buescher, Bell, and Barry found that the tissue uptake of selenium 75 was quite high for such a minor element in terms of total body composition. The swine rations contained 0.6 p. p. m. of selenium and therefore were essentially average in composition for swine diets.

The liver, while showing less selenium per gram of fresh tissue, contained more selenium than any other organ. The kidney contained most selenium on a per gram basis, but the content was highly variable. In terms of the selenium content in the total tissue, the organs in descending order were liver, lung, kidney, heart, and spleen.

This report on the effect of calcium upon the absorption of selenium and its subsequent deposition in the tissues is very interesting and suggests that selenium is not appreciably affected by the dietary calcium level. The adverse effect of high calcium upon diet consumption and consequent weight increase in pigs was reflected in the concentration of selenium in various tissues. It should be pointed out that this work uses selenium 75 as a tracer and does not include values for total selenium. While the tracer studies may reflect the actual utilization of selenium as it occurs in the diet, there is always the possibility that selenious acid, in which form the isotope was administered, may be metabolized somewhat differently from the selenium as it occurs bound in various compounds which occur in feed.

While very suggestive and of particular interest in view of the increasing attention being given to selenium, the need for total selenium values as well as tracer values cannot be ignored and suggests the need for future investigations with this element and with other dietary constituents which may influence its utilization.

SODIUM CHLORIDE AND MYOCARDIAL INFARCTS IN RATS

The drinking of 1 to 2 per cent saline increased the incidence of myocardial and renal infarcts and the degree of deposition of fat in the aortas of rats on an atherogenic diet.

The nutritional requirements of the rat are similar to those of man. In addition, its small size, short life span, and omnivorous eating habits add to its possible value as an experimental animal in the study of atherosclerosis.

The resistance of the rat to the development of lipid-containing arterial lesions has been overcome by the prolonged feeding of high fat diets, according to the report of R. W. Wissler, M. L. Eilert, M. A. Schroeder, and L. Cohen (*Arch. Path.* **57,** *333 (1954)*). The incidence of such lesions was greatly increased by the addition of renal damage and the administration of sodium chloride along with desoxycorticosterone acetate.

More recently, it has been demonstrated that dietary production of myocardial infarcts required another factor, sodium cholate, added to an already complicated diet (W. A. Thomas and W. S. Hartroft, *Circulation* **19,** *65 (1959)* and G. F. Wilgram, *J. Exp. Med.* **109,** *293 (1959)*).

Excessive intake of sodium chloride results in hypertension and is associated with an increased incidence of atherosclerosis in man. The present report by R. Belliveau and M. E. Marsh (*Arch. Path.* **71,** *559 (1961)*) deals with the influence of sodium

chloride on the production of atherosclerosis and on the incidence of myocardial and renal infarcts in rats.

Albino rats six to eight weeks of age were divided into four groups and fed an atherogenic diet with either tap or saline water. The saline concentration was 1 per cent in Group 2 and 2 per cent in Group 4. Animals were weighed initially and weekly. The food and drink were given ad libitum. Blood pressures were measured at intervals. Controls were selected at random from the colony and fed a stock ration. The experiment lasted 19 weeks.

The diet included 20 per cent casein, 20 per cent sucrose, 2 per cent sodium cholate, 5.3 per cent cholesterol and 40 per cent butter, but no propylthiouracil.

The heart and kidneys were examined grossly for infarcts; many other organs were also examined. Utilizing blocks of tissue from the vicinity of the infarcts, frozen sections of heart and kidneys were prepared and stained with Oil Red O. Serial sections were obtained at regular intervals to demonstrate occlusion of arteries. The diagnosis of infarction required fulfillment of three criteria: a grossly visible, sharply outlined area of necrosis, absence of diffuse areas of necrosis, and demonstration of the region of occlusion in the nutrient artery.

The animals gained weight on the atherogenic diet with either tap water or 1 per cent saline, but lost weight when 2 per cent saline was given.

The arterial walls of all animals given the atherogenic diet showed fatty deposition. The lesions were localized chiefly around the orifices of the intercostal arteries and branches of the aorta. The amount of deposited fat increased demonstrably when 1 per cent saline was given but no fatty deposits were found in aortas of animals fed the stock diet. No myocardial or renal infarcts occurred in rats receiving the stock diet or in those given the atherogenic diet with tap water or 1 per cent saline. In a second tap water group, one rat developed a renal infarct and another a myocardial infarct. In Group 4 (1–2 per cent saline) 11 rats had six renal infarcts and ten myocardial infarcts.

The recent and old renal and myocardial infarcts resembled those seen in man. Thrombi were readily found occluding the nutrient artery where kidney infarcts occurred. Arterial narrowing by fat but no thrombosis was found in serial and step sections of the heart. The distribution of fat varied within an artery and from artery to artery.

Hypertension was not demonstrated in this short term experiment, but assuming that anesthesia was employed, hypertension may have been masked. In any event, there was no significant difference in average initial and final blood pressures among groups.

The amount of fat accumulating in arterial walls of animals fed a high fat diet increased as a result of salt ingestion. Infarction resulting from occlusion of the arteries either by narrowing due to fat accumulation or thrombus was promoted by salt. The mechanism is unknown.

In a recent study reported by Thomas, Hartroft, and R. M. O'Neal (*J. Nutrition* **69,** *325 (1959)*) the addition of 1 per cent NaCl to a thrombogenic diet containing propylthiouracil resulted in no infarcts, but no animal survived longer than two months. However, in this study the incidence of myocardial infarcts (23 per cent) in rats receiving the thrombogenic diet and surviving two months, compared with the incidence in rats receiving three times as much salt mixture (incidence 38 per cent), suggests that minerals other than sodium chloride may have caused the rise in incidence of infarcts. Clearly the relationship of minerals to experimental arterial disease merits further study. Furthermore, the blood and tissue lipids should be analyzed in this type of experiment and the blood pressure should be measured at closer intervals.

DIETARY FATS AND ATHEROSCLEROSIS AND THROMBOSIS IN THE RAT

Saturated fatty acids are thrombogenic while linoleic acid in conjunction with saturated fatty acids is atherogenic in the rat.

Experimental evidence suggests that, along with other variables, unsaturated fats can be atherogenic and saturated fats thrombogenic in rats, rabbits, chicks, and human beings. High fat diets of butter, lard or hydrogenated fat plus cholesterol, cholic acid, and thiouracil produce myocardial infarcts and thrombosis in rats (W. S. Hartroft and W. A. Thomas, *J. Am. Med. Assn.* **164,** *1903* (*1957*)). Bleeding, coagulation, and prothrombin times are shortened and platelet counts are elevated by adding saturated fat to rabbit diets (*Nutrition Reviews* **18,** *246* (*1960*)).

Feeding unsaturated fats and wheat germ to chicks, however, increases serum cholesterol deposits in the aorta (*Nutrition Reviews* **18,** *146* (*1960*)). C. J. P. Böttcher *et al.* (*Lancet* **1,** *1378* (*1960*)) found that human atherosclerotic plaques have a high content of an unsaturated fat, linoleic acid; the content increases with the severity of the disease.

Linoleic acid and saturated fats also behave differently in cholesterol metabolism. Linoleic acid increases the synthesis and breakdown of cholesterol while saturated fats have an opposite effect (*Nutrition Reviews* **18,** *91* (*1960*)).

G. A. Gresham and A. N. Howard (*Brit. J. Exp. Path.* **41,** *395* (*1960*)) noted that atherosclerosis or thrombosis could be selectively produced by feeding rats diets containing different proportions of fat. Butter, cholesterol, cholic acid, and thiouracil produced thrombosis but substituting arachis oil for butter produced atherosclerosis. Since butter differs from arachis oil in containing large quantities of saturated fatty acids and small amounts of linoleic acid, the authors investigated the effects of diets containing different fatty acid compositions on the production of atherosclerosis or thrombosis and myocardial infarction in the rat (*Ibid.* **42,** *166* (*1961*)).

The animals were fed a diet of 40 per cent fat, 5 per cent cholesterol, 2 per cent cholic acid and 0.3 per cent thiouracil. The diet also contained 17 per cent sucrose, 20 per cent casein, vitamins, salts, cellophane flaked film, magnesium oxide, and inositol. The animals remained on the diet until death. The rats were divided into groups of five to ten and the fat content of the diet was varied in each group.

Rats fed butter, beef fat or hydrogenated arachis oil showed a 50 per cent incidence of thrombosis in the heart, aorta or coronary arteries. Myocardial infarctions occurred in eight of 38 animals. Many lipoid filled macrophages were found but no atherosclerotic changes were seen in the aorta.

Rats fed arachis oil showed an 88 per cent incidence of atherosclerotic plaques in the proximal aorta and coronary arteries but no thrombi. Rats fed maize oil developed both atherosclerosis and thrombosis. Lipoid filled macrophages were rare in the arachis and maize fed animals.

A combination of atherosclerosis, thrombosis, and myocardial infarction was produced in groups fed mixtures of butter and arachis oil, butter and methyl linoleate or arachis oil followed by butter.

Atherosclerosis was not produced in rats fed synthetic glycerides of trilinoleate, mono-oleate distearate, di-oleate monostearate or tri-oleate.

Thrombosis occurred in rats fed mono-oleate distearate and di-oleate monostearate. Rats fed trilinoleate or tri-oleate did not develop thrombi. Survival time was decreased in all rats fed synthetic glycerides.

The results indicate that the type of fat fed determined whether atherosclerosis or thrombosis and myocardial infarction would

occur. Arachis oil, containing large quantities of linoleic acid, produced atherosclerotic lesions similar to human atherosclerotic plaques. When linoleic acid was removed from arachis oil by hydrogenation, atherosclerosis was absent but thrombosis occurred.

Fats containing chiefly saturated fatty acids produced thrombosis and myocardial infarctions. This was true for butter and beef fats as well as for synthetic glycerides. The occurrence of thrombosis in rats fed synthetic glycerides was correlated with the amount of saturated fatty acid in the molecule. Mono-oleate distearate was most effective while tri-oleate and trilinoleate were ineffective in producing thrombi.

Feeding combinations of linoleic acid and saturated fatty acids caused atherosclerosis and also thrombosis and myocardial infarction.

A certain amount of saturated fatty acid was necessary for linoleic acid to produce atherosclerotic lesions, since feeding glyceryl trilinoleate alone was ineffective.

Maize oil produced a combination of atherosclerotic and thrombotic changes despite a fatty acid content similar to arachis oil. The authors speculate that this difference between the effects of maize and arachis oil is due to a different type of linkage of glycerides in the two oils.

The concept that saturated fatty acids produce thrombosis and myocardial infarcts while an unsaturated fatty acid, linoleic acid, with small amounts of saturated fat produces atherosclerosis is suggested by the data. Bleeding, clotting, and prothrombin times as well as platelet counts and concentration of linoleic acid in serum and plaques in the animals studied would be helpful in evaluating the hypothesis. The relationship between linoleic acid and saturated fatty acids is interesting. Both must be included in the diet in order to produce atherosclerosis. How are these two facts related? Does the addition of saturated fat increase absorption of linoleic acid in the gut? Perhaps the different metabolic effects of the two types of fat on cholesterol metabolism is involved.

The role of cholesterol in the study was not discussed. It was necessary, however, to include cholesterol as well as cholic acid and thiouracil in the diets of all rats in order to produce lesions.

Further studies on the problem of selective production of atherosclerosis versus selective production of thrombosis are indicated.

INTESTINAL CONVERSION OF C^{14}-CAROTENE TO VITAMIN A

In the presence of specific bile salts, the intestine of rats effectively converted intraduodenally administered, labeled β-carotene into vitamin A ester while vitamin A alcohol, retinene, and acidic compounds showed little radioactivity.

Although such organs as the liver can convert well-dispersed β-carotene into vitamin A, the intestine is normally the major site for this conversion (*Nutrition Reviews* **6,** *92, 281 (1948)*; **7,** *169 (1949)*; **12,** *23 (1954)*; **13,** *123 (1955)*). The mechanism is not understood. Prior conclusions are based on measurements such as growth, liver vitamin A, blood or lymph vitamin A, and nutritional balance; all of these are affected by several variables such as stability and rates of absorption, transport, turnover, and the like. Using C^{14}-labeled β-carotene, J. A. Olson (*J. Biol. Chem.* **236,** *349 (1961)*) has attempted a more direct study of β-carotene absorption and its conversion to vitamin A by rat intestine.

β-carotene uniformly labeled with C^{14} was isolated from *Chlorella pyrenoidosa* cultured in the presence of $C^{14}O_2$. A "stock-solution" of labeled carotene was isolated which contained 71 per cent of its radio-

activity as β-carotene. Immediately prior to each experiment, carrier β-carotene was added, the solution chromatographed on deactivated alumina, and the β-carotene band recovered. This minimized "artifacts" from the oxidation of β-carotene and assured a preparation in which β-carotene carried essentially all of the label.

Rats which had been fasted for two to six hours were anesthetized with ether; a section of the intestine was then ligated. A suspension of labeled β-carotene was injected into the ligated intestinal loop. The wound was closed with clips and the animal returned to a cage until a selected time up to five hours after operation at which time it was reanesthetized and the intestinal loop removed, opened, and rinsed thoroughly with saline. Lipid extracts were prepared from the contents of the intestinal lumen, the intestinal wall, and the liver. Following the addition of carrier β-carotene, vitamin A palmitate, retinene, and vitamin A alcohol to minimize artifacts, the lipid extracts were chromatographed on deactivated alumina and eluted with hexane and hexane-acetone. Radioactive fractions which were characterized in order of elution were β-carotene, vitamin A ester, retinene, and vitamin A alcohol. An uncharacterized polar fraction was also eluted with acetone.

Conversion of the C^{14}-carotene to vitamin A proceeded well during ether anesthesia. The disappearance of radioactive carotene from the intestinal loop approximated first order kinetics with a half-time of 0.83 hours. A peak of radioactivity occurred in the intestinal mucosa at one hour while in liver it plateaued at three to five hours.

In the intestinal mucosa vitamin A ester, which reached a peak at one hour, and β-carotene, which varied erratically, accounted for a very large share of the total radioactivity. Much smaller amounts of label occurred in retinene, vitamin A alcohol, and the terminal polar fraction, in that order. Most of the β-carotene was believed to be strongly adsorbed to the intestinal wall, because zero time controls showed similar concentrations and cyanide failed to have an effect. Excluding the β-carotene, vitamin A ester accounted for 81 to 98 per cent of the radioactivity in the vitamin A derivatives of intestine. None of the labeled vitamin A derivatives was found in the intestinal lumen.

In liver, vitamin A ester was always the most abundant labeled component. β-carotene showed a peak at one hour. Vitamin A alcohol was labeled to a considerably greater extent in liver than in intestinal mucosa, but much less than the ester. Retinene and the terminal polar fraction were virtually devoid of radioactivity throughout the five hour period.

Because labeled vitamin A ester was maximal in the intestinal mucosa at one hour and accounted for a high proportion of the radioactivity, it was used as a measure of β-carotene cleavage to vitamin A. Increased dosages of β-carotene caused increased amounts of labeled vitamin A ester in the mucosa. However, above 50 to 80 μg of β-carotene, the extent of increase was much less.

One important discovery was the rather specific requirement for bile salts. In bile duct-ligated rats, crude bile, glycocholate or cholate were effective but deoxycholate, ionic detergents such as sodium lauryl sulfate, or Tween 20 (polyoxyethylene sorbitan monolaurate) alone did not facilitate the conversion. Bile salts such as glycocholate appeared to have a more specific function than that of general emulsifying agents. They were postulated to enhance absorption of the β-carotene into the intestinal cells.

The upper one-third of the intestine was most active in vitamin A ester formation from β-carotene and the middle one-third usually less active. The lower one-third had little ability to carry out the conversion. This effect may relate both to lack of functional bile salts and to the inability of the cells to convert absorbed carotene.

Olson postulated that the uptake of β-carotene was the rate-limiting step in the over-all conversion, because (1) the amount in the intestinal wall was small compared to vitamin A ester, (2) possible intermediates such as vitamin A alcohol and retinene did not accumulate, and (3) the uptake of vitamin A from the lumen and its esterification were rapid compared to β-carotene uptake. In the absence of bile, carotene absorption is probably even more limited. Transfer of vitamin A ester to the lymph is also slow.

These studies to date give little conclusive information on the mechanism of the conversion of β-carotene to vitamin A. Two hypotheses are: the direct central cleavage hypothesis to yield two molecules of vitamin A (*Nutrition Reviews* **6,** *281 (1948)*) and the β-oxidation mechanism to yield one molecule of vitamin A (*Nutrition Reviews* **14,** *157 (1956)*). In the former, the radioactivity of the vitamin A ester would equal the radioactivity of the C^{14}-carotene disappearing. In the latter, vitamin A ester would account for 50 per cent of the labeled β-carotene disappearing and an equal amount would have been in short chain, probably acidic, oxidation products. About 40 per cent of the label could not be recovered from the intestine and its contents, presumably because of transport from the intestine and the formation of metabolites not extracted by lipid solvents. Therefore, no definite conclusion could be drawn. Without considering transport, intestinal vitamin A ester accounted for 40 to 50 per cent of the β-carotene disappearing. Appreciable quantities of nonpolar or acidic radioactive products did not accumulate, but could have been carried from the intestine. This possibility seems unlikely since others have shown that labeled carotene does not yield much $C^{14}O_2$. Olson, therefore, favors the central cleavage hypothesis.

This work represents a new approach to the problem of carotene to vitamin A conversion. If specific radioactivities of the compounds could have been evaluated, it may have been possible to resolve whether or not retinene and vitamin A alcohol are intermediates in the conversion of β-carotene to vitamin A ester as postulated. However, important discoveries were made and with further refinements the method has promise of elucidating the mechanism of the carotene-vitamin A conversion.

FATTY ACIDS AND RIBOFLAVIN IN THE METABOLISM OF VITAMIN B_6

Supplementary riboflavin or flavin mononucleotide prevented a decrease in liver pyridoxal phosphate and pyridoxine phosphate oxidase caused by feeding rats a high fat diet or the sodium salts of several fatty acids.

The close relationships between vitamin B_6 and fat metabolism are well supported (*Nutrition Reviews* **12,** *186 (1954)*), although uncertainty still exists concerning the mechanisms by which vitamin B_6 and fat metabolism are linked. Vitamin B_6 may function either directly as a coenzyme or only indirectly through its role in amino acid metabolism, especially in the synthesis of the nitrogenous bases of the phospholipids.

Among the relationships of vitamin B_6 and fat metabolism are the observations of Y. Kotake and his associates (*Nutrition Reviews* **14,** *183 (1956)*) who found that tryptophan, when fed together with the sodium salt of a fatty acid (butyric, palmitic, oleic or stearic acid), increased the urinary excretion of xanthurenic acid significantly more than did tryptophan alone. The injection of pyridoxine, however, reduced considerably the excretion of

xanthurenic acid in the rats fed tryptophan and sodium butyrate. These results suggested that the metabolism of the fatty acid increased the need for vitamin B_6 and made it less available for the metabolism of tryptophan.

A possible explanation for these results has been suggested by I. Nakahara, Y. Watanabe, Y. Morino, and Y. Sakamoto (*J. Biochem. (Japan)* **49,** *343 (1961)*). They speculated that a high fat intake might make less riboflavin available for its function as a component part of pyridoxine phosphate oxidase, a flavoprotein enzyme which catalyzes the oxidation of pyridoxine phosphate to pyridoxal phosphate (H. Wada *et al., Proc. Japan Acad.* **35,** *299 (1959)*). This speculation was based on evidence that diets high in fat increase the need of the rat for riboflavin (G. J. Mannering, M. A. Lipton, and C. A. Elvehjem, *Proc. Soc. Exp. Biol. Med.* **46,** *100 (1941)*). It was further postulated by Nakahara and co-workers that a decrease in the level of pyridoxine phosphate oxidase would decrease the amount of pyridoxal phosphate available for the metabolism of tryptophan, even in the presence of adequate dietary pyridoxine. Thus, because of a cellular deficiency of pyridoxal phosphate, xanthurenic acid excretion would increase—an indication of vitamin B_6 deficiency.

In the present studies of Nakahara and his associates sodium butyrate, sodium caproate, palmitic acid, stearic acid, and linoleic acid (0.3 g.) were given orally to young albino rats (body weight 120 to 140 g.) which had been fasted for 12 hours. At various intervals after feeding the fatty acids, the livers were analyzed for pyridoxal phosphate, pyridoxine phosphate oxidase, pyridoxal kinase, and pyridoxal phosphate phosphatase. The latter two enzymes were measured to determine whether more than one of the enzymes of vitamin B_6 metabolism were affected by the administration of these fatty acids.

When sodium butyrate was fed, the levels of pyridoxal phosphate and the activity of pyridoxine phosphate oxidase decreased within one hour, reached minimum values in four hours, and returned to normal levels after ten hours. Liver riboflavin also decreased slightly after four hours. No changes were observed in the activities of pyridoxal phosphate kinase or pyridoxal phosphate phosphatase. Feeding caproic, palmitic, and stearic acids also caused decreases in pyridoxal phosphate and pyridoxine phosphate oxidase comparable to the decreases observed with sodium butyrate. When linoleic acid was fed, however, liver pyridoxal phosphate and pyridoxine phosphate oxidase remained normal. When 100 μg. of riboflavin were injected at the time of feeding the fatty acids, no decrease occurred in pyridoxine phosphate oxidase, and there was only a slight decrease in pyridoxal phosphate.

The effect of riboflavin deficiency on liver pyridoxal phosphate and pyridoxine phosphate oxidase activity was also measured in rats fed a riboflavin deficient or riboflavin supplemented diet for two weeks. The composition of this diet was not given. In the deficient rats, some decrease occurred in both the concentration of pyridoxal phosphate and in the activity of the enzyme.

In another experiment, the effect of the dietary level of fat on the concentration of liver pyridoxal phosphate and pyridoxine phosphate oxidase was tested. A high fat diet (30 per cent butter) and a low fat diet (10 per cent butter) were fed for one to three weeks. The remainder of the diets consisted of 20 per cent casein, 6 per cent McCollum's salts, 4 per cent agar, and a B-vitamin supplement. Folic acid and vitamin B_{12} were not included in the vitamin supplement. Starch was the carbohydrate, and the percentage of butter increased at the expense of starch.

The activity of the enzyme was decreased after one week in the rats fed the high fat diet and continued to decrease at a comparable rate for the next three weeks. The

concentration of pyridoxal phosphate was unchanged in the first week, decreased slightly in the second week, and then decreased more rapidly in the third and fourth weeks. After four weeks, the concentrations of both pyridoxine phosphate oxidase and pyridoxal phosphate were approximately 50 per cent of the initial values. No values for the first two weeks were given for the rats fed the 10 per cent fat diet; effect of the transfer from a stock diet to the 10 per cent fat diet therefore cannot be evaluated. The rats fed the 10 per cent fat diet for three weeks, however, had higher levels of both pyridoxal phosphate and the enzyme in the liver than did the rats fed the 30 per cent fat diet.

To test whether additional riboflavin as flavin mononucleotide would prevent the decline in pyridoxal phosphate and pyridoxine phosphate oxidase activity in the rats fed the high fat diet, one group of rats fed the high fat diet and a corresponding group fed the low fat diet were injected daily for one week with 200 μg. of flavin mononucleotide. This treatment had no effect with the low fat diet, but increased the pyridoxal phosphate content and the pyridoxine phosphate oxidase activity in the group fed the high fat diet. No values were given for liver riboflavin. The authors concluded that, despite the riboflavin present in the diet (6.6 μg. per g.), the ingestion of a high fat diet caused a relative deficiency of riboflavin with a corresponding decrease in the activity of the flavin enzyme pyridoxine phosphate oxidase. This decrease then caused a secondary decrease in liver pyridoxal phosphate because of the inability to form pyridoxal phosphate from dietary pyridoxine.

These interesting results suggest another indirect pathway by which fat metabolism can affect the utilization of vitamin B_6. They also provide an example of how the utilization of one vitamin may depend upon the availability of another vitamin (*Nutrition Reviews* **19,** *122 (1961)*).

The observation that the feeding of linoleic acid did not decrease the levels of pyridoxal phosphate and pyridoxine phosphate oxidase as did the other fatty acids is very intriguing. The disparate results with linoleic acid may be related to its role as an essential fatty acid. On the other hand, no other unsaturated fatty acids, such as palmitoleic acid or oleic acid, were fed. The metabolism of any unsaturated fatty acid undoubtedly involves some steps different from those concerned in the metabolism of saturated fatty acids.

It is hoped that future work will show whether the results with linoleic acid apply to unsaturated fatty acids which are not essential fatty acids. If the effects reported here should be specific for linoleic acid, in contrast to other unsaturated fatty acids, they would suggest another explanation for the observations suggesting that essential fatty acids "spare" vitamin B_6 (T. Sakuragi, *J. Am. Oil Chem. Soc.* **36,** *251 (1959)*).

EXPERIMENTAL ATHEROSCLEROSIS IN DOGS WITH AORTIC GRAFTS

Cholesterol-fed hypothyroid dogs developed aortic lipid deposits. These were more severe in lyophilized homograft segments and were modified by administration of unsaturated fatty acids, estrogen or sitosterol.

The effects of dietary fats or of various fatty acids upon atherosclerosis and cholesterol metabolism have been reviewed (*Nutrition Reviews* **15,** *1, 17 (1957)*; **16,** *81 (1958)*; **18,** *281 (1960)*; **19,** *117 (1961)*). The effect of plant sterols on cholesterol absorption and atherosclerosis has been discussed (*Ibid.* **15,** *45 (1957)*; **18,** *26 (1960)*).

G. L. Jordan, Jr., D. M. Chapman, M. E. De Bakey, and B. Halpert have published an article entitled "The Effect of Linoleic and Linolenic Acid, Estradiol Valerate and Beta Sitosterol upon Experimentally Induced Hypercholesterolemia and the Development of Lipid Changes in the Aorta of Dogs" (*Am. J. Med. Sci.* **241,** *710 (1961)*).

The authors studied 20 dogs in which a segment of abdominal aorta had been surgically replaced by a lyophilized homograft. Since they had found homografts to be more susceptible to lipid deposits than the host aorta, a comparison between more and less susceptible portions was possible in each animal. Each dog was given 1 mc. of radioactive iodine per kg. body weight "to ablate the thyroid." The basal diet consisted of 38 per cent horse meat, 57 per cent "kibbles," and 5 per cent cholesterol. In addition to cholesterol the daily ration contained 43 g. saturated animal fat and 108 g. protein. The intake totaled 1,951 calories.

Group I served as a control to three experimental groups. Dogs of group II were fed 60 g. of an emulsion of unsaturated fatty acids consisting of linoleic acid with a "lesser" amount of linolenic acid and containing vitamin B_6. Another group was given 10 mg. of estradiol valerate per dog each week. The diet of group IV was supplemented by 45 g. of beta sitosterol per dog per day.

One animal in each group died in less than 15 weeks and was excluded from the study. The remaining four in each group survived 22 to 40 weeks.

The serum cholesterol concentrations (average of weekly determinations) in the control dogs were 763, 779, 878, and 934 mg. per 100 ml. respectively. Some atheromatous change developed in each and was "four plus" in both graft and host aorta of one animal.

Of the group fed the fatty acid emulsion, four plus changes were noted in the two animals with average cholesterol levels of 1,421 and 1,314 mg. per 100 ml. (However, the photograph and caption indicate only "occasional" plaques in the host aorta of the latter dog.) The remaining two animals, with average cholesterol levels of 791 and 634 per 100 ml. respectively, had two plus changes in the graft and none in the host vessel.

The four dogs given estradiol valerate had average serum cholesterol levels of 504, 604, 655, and 1,056 mg. per 100 ml. respectively. Two animals had no aortic atherosclerosis, one had two plus lesions in the aorta and three plus in the graft and one had three plus in both segments.

In the group treated with beta sitosterol the average serum cholesterol levels were 324, 370, 408, and 439 mg. per 100 ml. respectively. No atherosclerosis occurred in graft or host aortas of these dogs. However, this group suffered from anorexia attributed to the sitosterol and were unable to consume the full ration. They therefore did not receive the full dose of beta sitosterol and their caloric and cholesterol intakes were lower than those of the control dogs.

In the present experiments, the linoleic and linolenic acid supplements neither lowered the serum cholesterol levels as compared with the controls nor protected against atherosclerosis; the reverse occurred. However, the authors point out that the dogs fed the fatty acid supplement received 540 calories per day more than did the control animals.

The dogs treated with estradiol valerate had average serum cholesterol concentrations somewhat lower than those of the control animals and had somewhat less lipid deposited in the aortas.

In the case of the sitosterol treated dogs, the authors believe that the low serum cholesterol levels and absence of aortic lipid deposits may have been partly or largely attributable to inadequate caloric and cholesterol intake, leaving the effect of the beta sitosterol impossible to evaluate.

Aside from the fact that the experimental

groups are small, the principal difficulty in evaluating the results lies in the fact that the different groups are not readily comparable. The survival time of individual dogs is not given and we do not know whether it differed significantly in the different groups. This fact is important to know in any chronic feeding experiment, for the difference in serum cholesterol levels or aortic lipid deposits may be largely accounted for by a difference in time available for their development.

More important, as the authors realize, the failure of the linoleic and linolenic acid supplement to protect against hypercholesterolemia and atherosclerosis is difficult to assess because of the lack of dietary control. Thus, the control dogs received a 1,951 calorie diet containing 43 g. of fat (387 calories) per day and are to be compared with dogs receiving the same diet plus 540 extra calories derived from fatty acids. The latter represents a 28 per cent caloric supplement and an increase of about 140 per cent in lipid intake. Although the authors evidently hoped such a supplement of unsaturated fatty acids would be protective, one cannot draw conclusions with such a disparity in the caloric and lipid intakes of the two groups. The effects of unsaturated fatty acids, and specifically of linoleic acid, on cholesterol metabolism are mixed: both synthesis and degradation of cholesterol are involved, so that there are factors at the same time tending to raise and to lower the serum level (*Nutrition Reviews* **18,** *91, 146, 244 (1960)*). Moreover, the serum cholesterol level does not necessarily indicate whether deposition in the aorta is occurring.

The anorexia of the sitosterol treated dogs caused such a difference in cholesterol intake between this group and the controls, as mentioned above, as to make the absence of atherosclerosis in this group probably attributable to inadequate food intake.

CADMIUM, A POSSIBLE DIETARY ESSENTIAL

Metallothionein, a protein which contains large amounts of cadmium and zinc, has been isolated from equine renal cortex; no biological function has yet been demonstrated.

The dietary essentiality of the majority of required mineral elements has been elucidated through the experimental use of diets restricted in a particular element or through the study of naturally occurring deficiency diseases. The first approach is somewhat limited because of the difficulties in preparing diets virtually free of the element in question. Most nutritionists believe that the essentiality of certain mineral elements may not presently be recognized.

A technique which has been used in recent years to indicate the possibility that a mineral element is essential in the diet has been the demonstration of a specific association, in the native state, of the metal with a biological macromolecule. An example is the association of molybdenum with the enzyme xanthine oxidase (*Nutrition Reviews* **11,** *250 (1953)*). J. H. R. Kagi and B. L. Vallee (*J. Biol. Chem.* **235,** *3460 (1960)*) recently reported the isolation from horse kidney of a cadmium-containing protein, which suggests that cadmium may have a biological function.

Cadmium has previously been shown to occur in tissues of animals, especially in the kidney (A. K. Klein and H. J. Wichmann, *J. Assn. Official Agr. Chem.* **28,** *257 (1945)*). Vallee found that horse kidney, especially the cortex portion, had an extremely high cadmium content. Kagi and Vallee therefore used equine kidney cortex as a starting material and, by following the selective accumulation of the metal in the

course of protein purification, isolated a cadmium-containing protein.

The protein purification procedure involved extraction with phosphate buffer, ethanol and chloroform precipitation, ammonium sulfate fractionation, and dialysis. The final preparation represented about 0.4 per cent of the initial dry weight of the kidney cortex or about 1 to 2 per cent of the total soluble protein. Although the product gave a single peak on ultracentrifugation, it was not homogeneous since it gave one major and two minor protein components when subjected to paper electrophoresis. Metal analyses of the fractions during purification showed the cadmium to increase 61-fold from 550 p.p.m. of the dry kidney cortex to 33,600 p.p.m. in the dried, purified protein. Zinc increased 35-fold from 192 p.p.m. to 6,720 p.p.m. Eight other metals either were eliminated or changed little in concentration during the purification.

The purified protein fraction contained 19 per cent of the total cadmium and 12 per cent of the total zinc of the kidney cortex. Variation among different preparations may have been due to the fact that the protein was not completely purified. Cadmium content of the final dried material for ten different preparations ranged from 2.3 to 4.1 per cent and zinc content from 0.41 to 0.76 per cent. Nitrogen content also varied (mean of 13.2 per cent) but was invariably low compared to most proteins. One preparation was analysed and found to contain 1 per cent hexosamine, 1 per cent hexose, and 0.75 per cent sialic acid. The protein was distinctive as it contained no detectable aromatic amino acid and was unusually high in sulfur (4 per cent) because of the large number of cysteine residues. In view of the high metal and sulfhydryl contents, Kagi and Vallee named the protein metallothionein. Both zinc and cadmium could be removed by dialysis at a low pH, but cadmium was held more effectively than zinc. Dialysis at neutral pH against solutions of silver nitrate or para-chloromercuribenzoate, both of which react with sulfhydryl groups, resulted in complete displacement of cadmium and of zinc from the protein. Dialysis against zinc ions did not affect the cadmium content; however, upon dialysis against cadmium ions, cadmium displaced the zinc. These and other findings indicated that the metals were probably bound to the protein through the—SH groups.

The occurrence of both cadmium and zinc in metallothionein is not surprising, due to the similarities in chemical properties of the two elements. Zinc is a well-established dietary essential the need for which can not be satisfied by cadmium (H. G. Day and B. E. Skidmore, *J. Nutrition* **33,** *27 (1947)*). Cadmium has previously been considered with primary reference to its toxicity. The amount of metal bound in metallothionein is much greater than for known metalloenzymes and approaches the metal content of a "storage" metalloprotein such as ferritin. Although limited attempts were made to demonstrate a catalytic function of metallothionein, none was evident except for a slight acceleration of the reaction normally catalyzed by carbonic anhydrase. Thus, a biological function of this new metalloprotein has not been demonstrated, but it was suggested that it might be involved in catalysis, storage, immune phenomena, or detoxication.

The authors fully realized that the occurrence of this cadmium-containing protein in kidney can only serve as a tenuous basis for a functional hypothesis. Even if the protein has some important biological function, it will remain to be demonstrated that cadmium is specifically required and that a deficiency syndrome can be produced before a dietary need for this element is established.

It is possible that cadmium is a nonfunctional "contaminant" of this protein as a result of cadmium ingestion by the

animal. Perhaps zinc is the normal functional component and it has been replaced by cadmium or possibly the—SH groups of the protein are normally not bound to metal. The occurrence of this cadmium-containing protein in ten different preparations from horse kidney may argue against this possibility.

With reference to a possible functional role for cadmium, it is worth noting the very great "toxicity" of this element when injected into animals. A single, small dose of a cadmium salt representing 0.04 millimoles of cadmium per kg. body weight produced permanent sterility in male rats, an effect which was prevented by simultaneous injection of comparatively large amounts of zinc (J. Pařízek, *J. Reprod. Fertil.* **1,** *294* (*1960*)). The suggestion was made that cadmium displaced zinc from certain molecular structures into which zinc is incorporated during spermiogenesis.

The suggestion of Kagi and Vallee that cadmium may have a biological function, however, is certainly worthy of further investigation.

EFFECT OF EXCESSIVE LYSINE AND LEUCINE INTAKES ON THE PREGNANT RAT

Pregnant rats were fed massive excesses of L*-lysine and* L*-leucine during ten days of the gestation period. The amino acid supplements produced depressing effects on litter survival, maternal weight gain, and fetal weight.*

Recently, A. L. Rubin *et al.* (*Circulation* **21,** *332* (*1960*)) and R. P. Lasser, M. R. Schoenfeld, and C. K. Friendberg (*N. Engl. J. Med.* **263,** *728* (*1960*)) have reported remarkable success in the control of refractory fluid retention states associated with advanced heart failure and Laennec's cirrhosis by administration of massive dosages of L-lysine monohydrochloride in conjunction with mercurial diuretics. The L-lysine monohydrochloride is used not as a nutrient, but as an agent to produce a hyperchloremic acidosis necessary for good response to the diuretic.

The dosage of amino acid salt is often 40 g. per day, administered in four equal portions. For comparison, the amount of L-lysine monohydrochloride required for nitrogen balance in adult man is 1 to 2 g. per day. Although some of their patients had received this therapy for months at a time, no adverse clinical effects were observed by these investigators beyond occasional mild diarrhea, a result probably to be expected when such large amounts of a strong electrolyte are given orally. Nevertheless, there is always a question of possible toxic effects even with apparently innocuous nutrients when large amounts are administered over prolonged periods. In this connection, a recent study by S. Q. Cohlan and S. M. Stone (*J. Nutrition* **74,** *93* (*1961*)) is of considerable interest, as it involved administration, both orally and intraperitoneally, of massive excesses of L-lysine and L-leucine to pregnant rats through a ten day portion of the gestation period.

Pregnant animals were fed amino acid-supplemented diets from the fifth to the fifteenth day of gestation at two protein (casein) levels, 9 per cent and 27 per cent. The amino acid supplement comprised 10 per cent and 25 per cent of the total diet at each protein level. The other components of the diet are not given. Food and water consumption was ad libitum.

Among the 15 control animals receiving 27 per cent protein diets, there were 15 litters; maternal weight gain averaged 60.2 g. and the average 20 day fetus weight was 4.6 g. The corresponding figures for the 10 per cent L-lysine supplemented group were

11, 48.4, and 4.4; for the 10 per cent L-leucine group 12, 41.6, and 4.5; for the 25 per cent L-lysine supplemented group 6, 7.0, and 3.4; and for the 25 per cent L-leucine group 8, 11.1, and 3.6. The amino acid supplements therefore produced, in animals receiving the high protein diet, moderate depressing effects on litter survival, maternal weight gain, and fetal weight at 10 per cent, and severe effects at 25 per cent, of L-lysine or L-leucine in the diet.

With the 9 per cent protein diets the effect of the amino acid supplements on litter survival was similar, but the depressing effect on maternal weight gain was much more severe, while the effect on 20 day fetus weight was very slight.

A trial was made of intraperitoneal administration at approximately the median lethal dose (MId/50) levels as determined by P. Gullino *et al.* (*Arch. Biochem. Biophys.* **58,** *255* (*1955*)). As was to be expected, maternal mortality was extensive and litter survival greatly reduced. Surprisingly, average weight of surviving fetuses on the nineteenth day was not reduced.

Despite these massive doses of amino acids, there were no instances of gross fetal malformations among any of the surviving litters in either the dietary or the intraperitoneal experiments.

The value of this contribution is unfortunately reduced by poor experimental design and inadequate data. The authors note that food acceptance was impaired within 24 hours after consumption of the amino acid-supplemented diet was begun, yet no data on food consumption are given. Maternal weight gain, litter survival, and fetal weight can all be affected by the quantity of food consumed. It is therefore essential, in studies of this kind, that pair-fed controls be used if any valid conclusions are to be drawn regarding the effect of a given supplement as opposed to the general effect of a reduction in total food intake.

The L-lysine supplement is referred to throughout as "L-lysine," and there is no indication that either the authors or the editors realized that the standard form of this supplement is the monohydrochloride salt, a strong electrolyte. It is extremely unlikely that free L-lysine was used, as this is a highly unpalatable amine base. L-leucine, on the other hand, is ordinarily used as the free amino acid. Assuming that the ordinary salt form of L-lysine was the supplement employed, it would be useful to be able to compare the results of this study with observations made using similar levels of other relatively innocuous electrolytes. This is not easily done, however, as observations on electrolytes at such high concentrations in the diet are very rare.

One such study is that by G. R. Meneely, R. G. Tucker, and W. J. Darby on chronic sodium chloride toxicity in the growing rat (*J. Nutrition* **48,** *489* (*1952*)), which involved use of various levels of sodium chloride up to a maximum of 9.8 per cent of the diet. Increasing impairment of growth and damage to the renal and vascular systems were observed as the level of salt in the diet increased. Water consumption was greatly increased, but food consumption was not significantly depressed until the percentage of sodium chloride was above 7 per cent. J. H. Meyer (*J. Nutrition* **52,** *137* (*1954*)) fed growing rats 15 per cent sodium chloride in the diet at casein levels ranging from 10 to 35 per cent. In contrast to the work of Cohlan and Stone, pair-fed controls were used. At 10 per cent casein, the four week weight gains were 48.3, 43.9, and 80.5 g. for experimental, pair-fed controls, and ad libitum-fed controls, respectively. Similarly, the corresponding data at 25 per cent casein were 122.9, 115.7, and 176.5 g. Hence, the pair-fed animals actually gained less weight than those consuming the 15 per cent sodium chloride diet.

A glaring example of poor description in the Cohlan and Stone report is the use of the phrase "10 or 25 per cent amino acid excess" to describe the level of supplementation

used. The implication to many would be that if the amino acid requirement is a given percentage of the diet, then 10 to 25 per cent excesses over that percentage would produce the effects reported here. Actually, of course, the amounts of L-lysine and L-leucine added were large multiples of the requirements in each case. For example, in the case of L-lysine, approximately ten to 50 times the requirement for growth was fed in the various experiments reported. The over-all results of the study indicate that these amino acids show the same low orders of toxicity in the pregnant rat as those observed by Gullino and co-workers in the fasted adult rat.

VITAMIN A AND PROTEINS IN BLOOD AND LIVER

Vitamin A is transported in the blood as a protein complex and the blood level may be reduced when the serum albumin level falls as a result of a protein deficiency.

Increasing evidence is appearing which indicates that vitamin A in both blood and liver is linked to protein. Evidence for the association of vitamin A with a protein in the liver comes from a study by S. Krishnamurthy, S. Mahavdevan, and J. Ganguly (*J. Biol. Chem.* **233,** *32 (1958)*). They showed that when livers of normal rats were homogenized, the vitamin A in the supernatant fluid was precipitated by a variety of protein precipitating agents. When the protein was precipitated with 50 per cent ammonium sulphate, the precipitate could be redissolved in water. Under these circumstances, the vitamin A also went into solution. Vitamin A could be extracted with lipid solvents only after denaturation of the proteins. These observations led Krishnamurthy and co-workers to suggest that in the liver vitamin A is linked to lipoproteins.

Not only is the vitamin A in the liver bound in some way to protein, but so is the vitamin which circulates in the plasma. It is worthy of note that as early as 1914 L. S. Palmer and G. H. Ekles (*J. Biochem.* **17,** *223 (1914)*) reported that carotenoid pigments could not be extracted from plasma with ether. The addition of ethanol to the ether permitted the extraction of these lipid pigments. Now it is apparent that they were correct when they suggested that the alcohol denatured the protein which released the plasma carotene.

There is still some question as to the protein fraction with which the vitamin A is associated. E. S. Erwin, T. R. Varnell, and H. M. Page (*Proc. Soc. Exp. Biol. Med.* **100,** *373 (1959)*) reported that in calves the vitamin A in serum was associated primarily with albumin. Electrophoretic fractionation showed no other peak which had vitamin A activity. About 75 per cent of the carotene was also associated with the albumin with the remainder equally distributed among the α_1, β and γ globulins.

In contrast to the above is the work of C. F. Garbers, J. Gillman, and M. Peisach (*Biochem. J.* **75,** *124 (1960)*). They suggested that the vitamin was not associated with albumin. When Garbers *et al.* gave isotopically labelled vitamin A to rats they found 67 per cent of the radioactivity in the electrophoretic peak corresponding to α_1 globulin, with the rest of it associated with other globulins.

Although there is some confusion as to the nature of the plasma protein with which vitamin A is associated, it is accepted that the vitamin occurs in the blood linked to a protein. Much of the conflict arising from this problem may stem from the use of blood from different species. There is the possibility that in one species vitamin A may be associated with albumin while in others it may be linked to one or more of the globulins. Such a possibility appears to exist for the serum esterases which show considerable variation in their distribution among the protein fractions of different

species (J. Paul and P. Fottrell, *Biochem. J.* **78,** *418 (1961)*).

Recent work suggests that the transport of vitamin A may be limited under certain conditions by the level of plasma protein. One of these conditions may well be a protein deficiency such as kwashiorkor.

When infants with kwashiorkor first arrived at the hospital in Guatemala City, they showed no change in blood vitamin A levels for as long as seven hours after a test dose of 75,000 μg. of the vitamin (G. Arroyave, F. Viteri, M. Béhar, and N. S. Scrimshaw, *Am. J. Clin. Nutrition* **7,** *185 (1959)*). After five days of therapy with skim milk during which the infants received practically no vitamin A, the tolerance test was repeated. Five hours after the test dose, the serum levels were 300 μg. per 100 ml. At that time, the investigators were not sure whether the improvement in the vitamin A tolerance was the result of an increase in absorptive capacity or due to the restoration of more nearly normal plasma protein levels.

A recent abstract (Arroyave, D. Wilson, H. Castellanos, and Scrimshaw, *Fed. Proc.* **20,** *372 (1961)*) confirms the improvement in vitamin A tolerance in the kwashiorkor infants during therapy. In this study, one group of children was given the test dose only after one or two weeks of therapy with a vitamin A-low diet. This precaution was taken to rule out the criticism that the earlier study was complicated by the possible delayed absorption of the vitamin A in the first test dose.

In the preceding abstract, Arroyave and co-workers claim that the increase in blood vitamin A levels following a test dose occurs only when the hepatic reserves of the vitamin were adequate. How the latter was determined is not stated. They further reported that the level of vitamin A in the plasma (in the fasting state presumably) is related to the concentration of plasma albumin ($r = 0.917$).

A study of the relationship between protein deficiency and the transport of vitamin A in the blood has been made by C. J. Friend and co-workers (*Brit. J. Nutrition* **15,** *231 (1961)*). A commercial weaning diet was fed to pigs removed at five days of age from the sow. After about two weeks, the pigs were put into individual pens and fed five diets differing in the levels of B vitamins and protein. The basal diet for all groups consisted of whole wheat, cooked beans, lucerne meal, salts, corn starch, and the fat-soluble vitamins. The pigs that were fed the 15 per cent protein diet had, at the age of 16 weeks, serum albumin levels of 0.4 g. per 100 ml. At the same age, the control pigs fed a normal stock diet had levels of 2.9 g. The fasting level of vitamin A in the blood of the protein deficient pigs was 25 to 35 I.U. per 100 ml., while that in the control pigs was 134 to 136. Even when the pigs receiving the low protein intake were supplemented with a single dose of 100,000 I.U. of vitamin A, the fasting level for the next three days was still 46 I.U.

Although no data were provided, Friend and co-workers indicated that the pigs receiving the large single dose of vitamin A had approximately five times as much of the vitamin in their livers as did the undosed animals on the same diet. The protein deficient pigs stored approximately 20 per cent of the test dose in their livers, whereas the normal animals stored 80 per cent.

There was no correlation between the level of vitamin A in the liver and that circulating in the blood. A similar observation had been made for rats by Ganguly and N. I. Kirnsky (*Biochem. J.* **54,** *177 (1953)*).

Although there was no correlation between the level of vitamin A in the liver and that in the blood, there was a significant correlation ($r = 0.76$) between the concentration of vitamin A and albumin in the serum of the pigs (Friend and co-workers, *loc. cit.*)

To further substantiate the thesis that protein deficiency may influence serum vitamin A levels, one pig was maintained on a thiamine-deficient diet containing ample levels of both protein and vitamin A. Although this animal suffered from inanition

and had low levels of vitamin A in its liver, its serum vitamin A was 140 I.U. per 100 ml. This normal level of vitamin A was consistent with a serum albumin level of 2.7 per cent which was the same as that of the control animals fed a commerical stock diet.

When isocaloric diets were fed to two pigs with one diet having a higher protein content than the other, the animals had approximately the same amount of vitamin A in their livers. There was, however, a much higher level of vitamin A in the serum of the animal receiving the high protein diet when compared with that for the animal on the low protein diet (157 I.U. vs. 44 I.U. per 100 ml.). The albumin levels in these two pigs were 2.6 and 0.6 per cent respectively.

In the pigs, as in the kwashiorkor children, recovery from the protein deficiency was associated with an increase in plasma vitamin A levels even though there was no increase in the dietary level of the vitamin. Here, too, it was demonstrated that supplementing the animals on the low protein diet with vitamin A increased the liver stores but had no effect on the serum levels.

These studies on the relation between protein intake and circulating vitamin A prove the dependency of the circulating vitamin A level on certain protein fractions in the blood. The exact protein fraction has not been firmly established. The work so far also suggests that in a protein deficiency the liver retains its ability to store vitamin A long after the serum has lost most of its capacity to transport the vitamin.

The above observations suggest that serum levels of vitamin A by themselves cannot necessarily be interpreted as an indication of a low vitamin A intake. Additional work should be done to establish the limits of serum proteins which must be present in serum before a vitamin A deficiency can be adequately diagnosed. This is especially true when the serum vitamin A level is low.

There are a number of questions that arise in attempting to fathom the physiological significance of these observations. What is the reserve in the serum vitamin A levels as far as transport is concerned? What is the rate with which the serum vitamin A is turned over? Is it possible that this rate may be decreased in a protein deficiency? Must there be a minimum vitamin A level in the plasma to permit the uptake of the vitamin by the cells? This would be especially pertinent in the case of the liver which can store the vitamin even though the serum shows no change when a supplement is given. Why does the liver retain its capacity to store vitamin A long after a protein deficiency has curtailed ability of the plasma to transport the vitamin? These and other questions that come to mind should stimulate future workers in this field, which is beginning to elicit a new interest on the part of both nutritionists and biochemists.

PATHOLOGIC AND PHYSIOLOGIC STUDIES IN CANINE ATHEROSCLEROSIS

Hypercholesterolemia, increased peripheral resistance, abnormal electrocardiograms and ballistocardiograms parallel the development of atherosclerosis. In remission, the ballistocardiogram and hypercholesterolemia return to normal.

Canine atherosclerosis is similar in many respects to human atherosclerosis (*Nutrition Reviews* **8,** *74 (1950);* **9,** *295 (1951);* **10,** *8 (1952)*). Plaques are found, early in the development of the disease, in the coronary and cerebral arteries.

In the dog, atherosclerosis is produced by feeding cholesterol and thiouracil or by thyroidectomy and vitamin D injection. Since viosterol causes kidney damage, the former method, although longer, is preferred for the dog.

A. N. Fedoseev (*Bull. Exp. Biol. Med. (USSR)* **50,** *1129 (1961)*) studied simul-

taneous pathologic and physiologic changes during the course of and after remission of canine atherosclerosis. Twelve animals were used; two served as controls and two were followed for six months after cessation of the atherogenic diet (*Ibid.* **50,** *1034 (1961)*).

Simultaneous feeding of cholesterol (one g. per kg. body weight per day for 120 days) and 6-methylthiouracil (1.5 g. for 60 days and 1.0 g. for 60 days) produced atherosclerosis. Two control dogs were fed 1.5 g. of 6-methylthiouracil daily for 90 days. Blood cholesterol was determined at least once every three weeks.

Blood pressure was taken from an exteriorized left common carotid artery. Pressure was determined by a rubber cuff attached to a sphygmomanometer. The pressures were taken each morning while the dog lay quietly and after exercise on a treadmill (6.5 km. per hour for five minutes). Pressures were recorded three months prior to and at least one month after cessation of the atherogenic diet.

Electrocardiograms (E.C.G.) and ballistocardiograms (B.C.G.) were done before, during, and after feeding the atherogenic diet (Fedoseev, *Bull. Exp. Biol. Med. (USSR)* **49,** *457 (1960)*).

The velocity of the pulse wave spread was measured also, the rate of transmission determined from the common carotid artery to the femoral artery. All animals were sacrificed for gross and microscopic examinations.

The weight changes of the dogs were not consistent. Some lost but most gained body weight after four months of atherogenic diet. The largest weight gain was 10 kg. The two control dogs gained 1 to 2 kg. Hypothyroidism was apparent in the experimental but not in the control group.

Blood cholesterol concentrations prior to the experiment ranged from 120 to 160 mg. per 100 ml. During feeding of the atherogenic diet the cholesterol concentration rose to six to eight times normal. The highest figure was 1,200 mg. per 100 ml. The majority of the dogs showed a rise in cholesterol concentration and a subsequent fall followed by another rise. The fall in blood cholesterol usually occurred in the second and third months. The blood cholesterol in the control dogs rose 50 to 100 per cent.

No persistent elevation of resting blood pressure was noted in any of the dogs. The cardiovascular systems in the two groups responded to exercise differently, however. The control dogs demonstrated a slight fall in blood pressure and a slight rise in pulse rate following exercise. The experimental group showed an elevation in both heart rate and blood pressure. Some of the dogs in the experimental group fell on the treadmill after only moderate exercise.

The E.C.G.'s of the control animals were normal. The E.C.G.'s of the dogs on the atherogenic diet, however, looked much like E.C.G.'s of humans with coronary insufficiency. Tachycardia, arrythmias, decreased voltage of QRS complex, inversion of t wave, and displacement of the S-T segment were seen. Signs of atrial-ventricular block and isolated p waves were also noted. Arrythmias were most common after exercise.

The B.C.G. demonstrated a reduction in total amplitude in the atherosclerotic dogs.

The conduction of the pulse wave was unchanged in all animals.

The dogs on the atherogenic diet demonstrated atherosclerotic plaques in small, medium, and large arteries. Larger arteries involved were the femoral, carotid, and those at the base of the brain. Yellowish areas representing fatty infiltration were seen in the aorta, but no plaques were found. Extensive lipoidosis was seen in the liver, spleen, and kidneys. The cardiovascular systems of the control dogs looked normal.

No correlation was found between hypercholesterolemia, weight change, and degree of atherosclerotic damage.

Two dogs were followed for six months after cessation of the atherogenic diet. Clinically, the dogs seemed healthy and lively. Lost weight was partly regained.

Hypercholesterolemia persisted for two months before returning to normal. The B.C.G. approached the predietary picture. Blood pressure, however, continued to rise after treadmill exercise and the E.C.G. remained abnormal, especially after exercise. The pulse rate response to exercise was not mentioned.

Pathologic findings were much less marked. The few atherosclerotic plaques found in the aorta, carotid, and other arteries contained little fat and were becoming scarred. Very little fat was noted in other organs. The thyroid glands looked normal. Sclerosis was noted, however, in cardiac muscle and liver.

These papers stress the concomitant physiologic and pathologic changes which seem to parallel each other. When the atherosclerotic process was most severe the blood cholesterol was high, the peripheral resistance elevated, and the E.C.G. and B.C.G. abnormal. The clinical condition was poor. Six months after cessation of the atherogenic diet, the animals' clinical condition improved and blood cholesterol and B.C.G. returned to normal. Pathologic findings were meager. The article supports the hypothesis that both physiologic and pathologic findings show remission from the atherosclerotic process when the atherogenic diet is withdrawn.

Some facts remain to be explained. Cardiac fibrosis occurred and abnormal E.C.G's persisted despite involution of the atherosclerotic process. The important hemodynamic response of increased peripheral resistance apparently also persisted. It is unfortunate that figures of blood pressure rise following exercise were not given and that this response was not investigated more thoroughly in all animals. Increased peripheral resistance can only be surmised. Obviously, stroke volume and cardiac output determinations are needed to evaluate resistance properly.

The study deserves to be repeated. These investigators, by simultaneous cholesterol and 6-methylthiouracil feeding, have shown that extensive atherosclerosis can be produced in the dog in half the time reported by earlier workers (*Nutrition Reviews* **8,** *74 (1950)*). Documentation of physiologic functions that change due to dietary manipulation and those that do not are well worth study. More sophisticated measures of hemodynamic responses are called for. The reason for the drop in blood cholesterol which occurs during the second and third month of the atherogenic diet seems another fruitful area for study.

TRIVALENT CHROMIUM AND GLUCOSE TOLERANCE

The active component of the dietary glucose tolerance factor is trivalent chromium; in the presence of small amounts of insulin, trivalent chromium increased in vitro the glucose uptake by adipose tissues from deficient rats.

The investigations which indicated the existence of a dietary glucose tolerance factor have previously been reviewed (*Nutrition Reviews* **17,** *50 (1959)*). Since then, the active ingredient has been identified as trivalent chromium and studies on its possible mode of action have begun.

W. Mertz and K. Schwarz (*Arch. Biochem. Biophys.* **58,** *504 (1955)*) noted that rats which developed liver necrosis as a result of consuming a Torula yeast diet showed a slow rate of removal of excess glucose from the blood and that this could be corrected by dietary means. Later they demonstrated that the glucose tolerance factor was distinct from factor 3 (a selenium compound) which prevented the liver necrosis (Schwarz and Mertz, *Ibid.* **72,** *515 (1957)*).

Later Schwarz and Mertz (*Arch. Biochem. Biophys.* **85,** *292 (1959)*) reported the identification of trivalent chromium as the active ingredient of the glucose tolerance

factor. During fractionation of sources of glucose tolerance factor such as defatted pork kidney, it became apparent that the factor showed cationic properties and was not destroyed by wet ashing. A number of mineral elements were then screened for activity (a total of 47 elements have been tested). Only mixtures containing trivalent chromium were active. Compounds such as sodium dichromate which contained chromium in other valency states were not active unless components were included to convert some of the chromium to the trivalent state. However, not all trivalent chromium compounds were active; those complexes which are very stable had little activity. Subsequently, chromium was identified in concentrates of the glucose tolerance factor.

The method used by Schwarz and Mertz to assay for glucose tolerance factor was to feed young rats a diet lacking this factor for four to five weeks. Glucose tolerance was measured after a 16 to 18 hour fast by measuring blood glucose at intervals for about an hour following the intravenous administration of 125 mg. glucose per 100 g. of body weight. Because the rate of decrease of the *excess* blood glucose was logarithmic, the glucose tolerance was expressed as the percentage decrease of *excess* blood glucose per minute. Diets which were deficient in the glucose tolerance factor, such as the Torula yeast diet, usually produced excess glucose removal rates of about 2 per cent per minute, whereas diets containing or supplemented with the factor produced rates above 4 per cent per minute.

Of great interest is the finding that diets need not necessarily be of the "semi-synthetic" type to be deficient in glucose tolerance factor (Mertz and Schwarz, *Am. J. Physiol.* **196,** *614 (1959)*). A natural diet composed of wheat, crude casein, whole milk powder, Wesson oil, calcium carbonate, sodium chloride, and fat-soluble vitamins and supplemented with kale (designated McCollum's wheat-casein diet) and a diet composed of homogenized table scraps contained the glucose tolerance factor. However, three widely used commercial brands of laboratory animal ration or dog food were deficient in the factor. These feeds were routinely used to produce rats which exhibited low rates of excess glucose removal. The reason that the stock diets, which undoubtedly were composed mainly of natural ingredients, were deficient in glucose tolerance factor is not known. The factor may have been absent from the ingredients or it may have been destroyed during processing or storage, perhaps because of incompatibility with other dietary ingredients.

In a recent paper Mertz, E. E. Roginski, and Schwarz (*J. Biol. Chem.* **236,** *318 (1961)*) described experiments designed to define more closely the site of action of trivalent chromium complexes (*i.e.* glucose tolerance factor). They studied the *in vitro* uptake of glucose and the incorporation of labeled glucose into fat by epididymal fat tissue of rats fed diets either deficient in or supplemented with glucose tolerance factor (as hexaurea chromium(III)trichloride).

Rats which received the deficient, commercial stock diet showed a mean excess blood glucose removal rate of 3.7 per cent per minute compared to 5.7 per cent per minute for those fed the chromium compound (1 mg. of chromium per 100 g. of diet). These values are considerably higher than those reported previously; however, it is recognized that many other factors influence glucose tolerance. When the same rats were sacrificed and glucose uptake by epididymal fat tissue was studied *in vitro* during a two hour incubation period, no difference was apparent unless 1 milliunit of insulin was added to each flask. In this case tissues from the rats fed the chromium compound showed a glucose uptake which was 67 per cent higher than tissues from those rats which received the unsupplemented diet.

In vitro addition of 0.1 μg of trivalent chromium per flask, as chrome alum (chromium potassium sulfate), to the fat tissues from unsupplemented animals produced a maximal increase in glucose uptake when 1

milliunit of insulin was also added. At lesser or greater amounts of insulin, the effect of chromium was much less and no effect of chromium was apparent at 0 or above 10 milliunits of insulin. When the amount of insulin was held constant at 1 milliunit per flask and the chromium additions varied, a maximal response of about 90 per cent increase in glucose uptake was observed at 0.01 to 0.1 μg of chromium. Thus, chromium was active at levels which are found in many natural materials. Chromium additions *in vitro* caused increased glucose uptake only if the rats had been "depleted" of glucose tolerance factor and did not raise uptakes above the "normal" value.

Of 13 other transition elements tested in the *in vitro* glucose uptake system, only manganese had activity, which was about 50 per cent that of chromium. However, manganese does not have glucose tolerance factor activity in the intact animal. It probably acted on a different step of glucose utilization, such as fat synthesis, than did chromium.

Chromium was also shown to enhance *in vitro* the incorporation by epididymal fat tissues of glucose-C^{14} into fat and to a lesser extent of acetate-1-C^{14} into fat. The effect on acetate incorporation was not completely prevented by omission of insulin but it was essentially eliminated by witholding unlabeled glucose from the medium. The primary effect of chromium was probably on the initial steps of glucose utilization.

The effect of chromium on glucose uptake by peripheral tissues was of sufficient magnitude to explain the effect of glucose tolerance factor on intact animals. Whether or not other sites are involved has not been ascertained. Although the mechanism of action of chromium is not known, its dependence on insulin is intriguing and Mertz, Roginski, and Schwarz suggested that the active site of chromium is close to that of insulin.

The over-all nutritional significance of trivalent chromium as a glucose tolerance factor is presently unknown. Mertz and Schwarz (*Am. J. Physiol.* **196,** *614 (1959)*) believe that the evidence is sufficient to designate this factor, and therefore presumably the element chromium, as an essential dietary ingredient. However, the only deficiency "impairment" shown to date is a slower clearance of excess glucose from the circulatory system. No growth defect or other effect on the well-being of animals has been demonstrated. Of great significance is the fact that diets known to be deficient in glucose tolerance factor are widely used in experimental work including that which involves carbohydrate utilization and metabolism. Schwarz's group has pointed out that, because some forms of chromium are inactive, an assay for chromium does *not* evaluate a diet for this factor; only a bioassay based on glucose tolerance suffices.

Much remains to be learned about trivalent chromium and its significance in nutrition, metabolism, and disease. However, this is an excellent example of a "chance" discovery of an important biological phenomenon and stresses again the importance of critical observation irrespective of the immediate goals of an experiment.

NOTES

Letter to the Editor

Dear Sir:

Referring to your discussion of Bitot's spots (*Nutrition Reviews* **19,** *104 (1961)*), there is a correlation between Bitot's spots and xerophthalmia, and often with nightblindness. In a series of thousands of cases of xerophthalmia, at the Eye Hospital, Jogjakarta, Indonesia, the presence of the spots was noted in two-thirds of the case histories; in the remainder I suspect that

it was often taken for granted by a busy ophthalmologist.

Bitot's spots also occur in chronic eye affections, when there is nothing suggesting xerophthalmia. A series of such cases in Indonesia has been described by Sie-Boeh-Lian (*Geneesk. Tijdschr. Ned. Indië* **77,** *3283 (1938)*).

I know also of genuine cases of xerosis conjunctivae, in which the spots were absent. Such would be the rule if there is marked destruction of the cornea.

This should not detract attention from the fact, that, at least regionally, Bitot's spots are a useful indicator of xerophthalmia. Despite the occurrence of spots of different origin, I would warn the practitioner or the village nurse to keep his eye on the toddler or the schoolchild in whom he chanced to detect Bitot's spots! They are the ones who on a fateful day are liable to present irreparable corneal damage.

Nor do I wholly agree with your description of the spots. They are situated in the shallow depression, next to the limbus corneae, which has been called the space of Bitot. This is the area constantly exposed during the wake period and it is triangular. In my experience the common type of Bitot spot is triangular too. It is more pronounced on the temporal side—especially so in cross-eyed children!—but it occurs regularly on the nasal side. Chronic or worn-out spots, in a stage of recovery show the characteristics you describe (*for macrophotographs see Int. Rev. Trop. Med.* **1,** *142 (1961)*).

Regarding serum vitamin A levels, I must stress the point that in certain child populations they are low, irrespective of the incidence of Bitot's spots and of xerosis conjunctivae or night blindness. In my experience a "normal" child may have 20 I.U. per cent; a child with xerosis 40 I.U. per cent. What in kwashiorkor happens with the serum albumin levels, occurs in xerophthalmia regarding serum Vitamin A.

I agree that Bitot's spots are correlated with poor nutrition in a general sense. Still I agree more with a statement of de Haas (*Geneesk. Tijdschr. Ned. Indië* **80,** *728 (1940)*) that "dystrophy and xerophthalmia go hand in hand, while the vicious circle is closed by infections." Neither full-blown xerophthalmia, nor the spots, can yet be regarded as the safe single symptom, indicating a lack of the one nutrient vitamin A.

H. A. P. C. Oomen
Royal Tropical Institute
Amsterdam, Netherlands

Chemicals In Food

Publication 887 of the National Research Council, Washington, D. C., is a recent report of the Food Protection Committee. It is entitled The Use of Chemicals in Food Production, Processing, Storage, and Distribution.

Presented is a discussion of some of the technologic reasons for the use of chemicals in the food industry, the problems associated with this use, the research undertaken by industry and government to solve these problems, and the legal measures established to ensure protection of the public by governing the use of chemicals in foods.

Every chemical used in food production and processing should serve one or more of these purposes: improve nutritional value, enhance quality or consumer acceptability, improve the keeping quality, make the food more readily available or facilitate its preparation.

In recent years there has been considerable discussion of the possibility that hazards may result from the use of additives in the production and processing of foods. There has been no justification for any of the exaggerated viewpoints that have been expressed, but it is true that many new chemicals have been and are being introduced and that it is well to examine the existing situation.

As our population grows and becomes increasingly urbanized, less and less agricultural land per capita and proportionately less of the population can be devoted to the production of food. To provide a constant, wholesome, and adequate supply of food

for this population, increased production per acre and per man, and increased reliance on protection of the food from deterioration during storage and distribution, will be required. Technologic and scientific applications will have to assume increasing roles in providing and protecting the food supply. New chemical aids to production, processing, packaging, and distribution will be among those applications.

Experience has amply demonstrated that chemical aids of this kind can be used safely and beneficially. Competent, strong regulatory agencies and a public spirited, ethical industry give confidence that they will be so used in the future.

Dietary Treatment of Familial Mediterranean Fever

A rare condition known as familial Mediterranean fever is characterized by recurrent episodes of pain in the chest and abdomen, fever, arthralgia, and a family history of the illness. The underlying metabolic defect of this disorder is unknown.

A chance discovery is reported by S. M. Mellinkoff, A. D. Schwabe, and J. S. Lawrence, *Arch. Int. Med.* **108,** *80 (1961)*). The authors, who were studying the effects of low fat diets on a number of patients, were told by one patient that he could induce an attack of fever and abdominal pain by eating pickles and bacon. The authors also learned of a patient and his father who had familial Mediterranean fever and who had benefitted from a low fat diet.

They were able to study eight subjects with this familial condition. They measured the quantities of lipids and cholesterol in the serum of these patients and then instituted a low fat regimen. They kept careful records of the number of attacks which occurred.

The frequency of attacks in this group of patients was reduced dramatically. The few attacks which did occur usually followed some violation of the prescribed diet. It is interesting to note that the values reported for serum cholesterol and total serum lipids prior to therapy were near the upper range of normal and that these decreased considerably following the dietary restrictions. Measurements also were made of urinary histidine and of etiocholanolone, but these results were inconclusive.

The authors concluded that this was a fascinating sequence of events, but hesitated to speculate upon the mechanism involved. They have not yet investigated the possibility that certain types of fat might precipitate this syndrome while others might not, nor have they determined whether the measurable aspects of lipid metabolism are in any way altered in patients with this inheritable syndrome.

It would seem logical to compare this condition with several others in which abdominal pain and lipids are related; for example, the syndrome of abdominal pain associated with hereditary hyperlipemia (B. C. Payne and A. M. Waldron, *Ann. Int. Med.* **49,** *1420 (1958)*), the fat overloading syndrome characterized by fever and abdominal pain following the administration of excessive quantities of fat emulsions intravenously (C. S. Alexander and L. Zieve, *Arch. Int. Med.* **107,** *514 (1961)*), and Whipple's disease in which a high fat diet may precipitate abdominal pain, fever, and arthralgia (J. A. Clifton, R. E. Bleiler, and H. P. Schedl, *Gastroenterology* **40,** *670 (1961)*). This report should stimulate further investigation.

Recent Books

Food Additive Control in the Netherlands. W. Meijer (Director, Foodstuffs Control Service, The Hague). Published by Food and Agriculture Organization of the United Nations, Rome, Italy. Pp. 41. Price $.50.

Technical Manual No. 8-501—Nutrition. (This manual supersedes TM 8-501, September, 1949.) Headquarters, Department of the Army. Pp. 133.

BOARD OF TRUSTEES

W. GARDNER BARKER, *President, Thomas J. Lipton, Inc.*

LEE S. BICKMORE, *President, National Biscuit Company*

*CLAUDE T. BISSELL, *President, University of Toronto*

*F. G. BOUDREAU, *President, Milbank Memorial Fund*

WILLIAM T. BRADY, *Chairman, Corn Products Company*

CHARLES S. BRIDGES, *President, Libby, McNeill & Libby*

G. BUITONI, *President, Buitoni Foods Corp.*

*LEROY E. BURNEY, *Alexandria, Virginia*

*O. C. CARMICHAEL, *Biltmore, North Carolina*

THOMAS P. CARNEY, *Vice President, Research, Development & Control, Eli Lilly and Company*

HAROLD W. COMFORT, *President, The Borden Company*

HERBERT C. CORNUELLE, *Pres., Dole Corp.*

W. T. CRIGHTON, *General Manager, Producers Creamery Company*

WESLEY M. DIXON, *Chairman and President, Container Corporation of America*

*LEE A. DUBRIDGE, *President, California Institute of Technology*

JOSEPH EICHBERG, *President, American Lecithin Co., Inc.*

*CONRAD A. ELVEHJEM, *President, University of Wisconsin*

THOMAS C. FOGARTY, *President, Continental Can Co., Inc.*

EDGAR J. FORIO, *Senior Vice President, The Coca-Cola Company*

*CLARENCE FRANCIS, *New York, New York*

T. R. GAMBLE, *President, Pet Milk Company*

DANIEL F. GERBER, *President, Gerber Products Co.; Vice Chairman, Executive Committee, The Nutrition Foundation, Inc.*

GEORGE A. GRAHAM, *Secretary Treasurer, Charles B. Knox Gelatine Co., Inc.*

JOHN A. GRAMMER, *Executive Vice President, Beech-Nut Life Savers, Inc.*

HENRY J. HEINZ, II, *Chairman, H. J. Heinz Co.*

*REV. THEODORE M. HESBURGH, C.S.C., *President, University of Notre Dame*

FRANK C. HILDEBRAND, *Vice President, General Mills, Inc.*

*HERMAN E. HILLEBOE, *Commissioner of Health, State of New York*

S. F. HINKLE, *President, Hershey Chocolate Corporation*

PORTER M. JARVIS, *President, Swift & Company*

*JAMES R. KILLIAN, JR., *Chairman, Massachusetts Institute of Technology, Chairman of Board of Trustees, The Nutrition Foundation, Inc.*

*CHARLES GLEN KING, *President, The Nutrition Foundation, Inc.*

*GRAYSON L. KIRK, *President, Columbia University*

R. NEWTON LAUGHLIN, *President, Continental Baking Co.*

J. PRESTON LEVIS, *Chairman, Owens-Illinois*

DAVID M. LEVITT, *President, DCA Food Industries Inc.*

R. G. LUCKS, *President, California Packing Corp.*

W. F. MCLEAN, *President, Canada Packers, Limited*

WILLIAM H. MCLEAN, *President of Chemical Division, Merck & Co.*

OSCAR MAYER, *Chairman of the Board, Oscar Mayer & Co.*

CHARLES G. MORTIMER, *Chairman, General Foods Corporation; Chairman, Executive Committee, The Nutrition Foundation, Inc.*

MILTON C. MUMFORD, *President, Lever Brothers Co.*

W. B. MURPHY, *President, Campbell Soup Co.*

H. EARLE MUZZY, *Vice Chairman, The Quaker Oats Co.*

WILLIAM F. OLIVER, *President, The American Sugar Refining Company*

W. W. PADDON, *President, Sunshine Biscuits, Inc.*

*THOMAS PARRAN, *President, Avalon Foundation*

*MAURICE PATE, *Executive Director, United Nations Childrens Fund*

PHILIP W. PILLSBURY, *Chairman, The Pillsbury Company*

J. G. PLEASANTS, *Vice-President in charge of Research and Development, The Procter & Gamble Company*

LLOYD E. RIGLER, *President, Adolph's, Ltd.*

LYLE C. ROLL, *President, Kellogg Company*

R. T. RYAN, *Executive Vice President, Welch Grape Juice Co., Inc.*

ELBERT M. SHELTON, *Secretary, The Baker Laboratories, Inc.*

VERNON STOUFFER, *President, The Stouffer Corporation*

ROY J. SUND, *President, American Can Company*

THOMAS E. SUNDERLAND, *President, United Fruit Company*

HENRY WEIGL, *President, Standard Brands, Incorporated*

J. HUBER WETENHALL, *President, National Dairy Products Corporation*

F. R. WILCOX, *General Manager, Sunkist Growers*

E. W. WILSON, *President, Armour & Company*

R. D. L. WIRTH, *President, Red Star Yeast and Products Co.*

H. J. WOLFLISBERG, *President, The Nestle Company, Inc.*

HORACE L. SIPPLE, *Secretary* JOHN HOLMES, *Honorary Trustee*

* Representative of the public

THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit, in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

Signed articles do not necessarily represent the opinion of the Editorial Staff.